



## DOCTOR OF HEALTH (DHEALTH)

### Can we Improve how we Diagnose Osteomyelitis in the Diabetic Foot?

Harman, Kim

*Award date:*  
2010

*Awarding institution:*  
University of Bath

[Link to publication](#)

## Alternative formats

If you require this document in an alternative format, please contact:  
[openaccess@bath.ac.uk](mailto:openaccess@bath.ac.uk)

Copyright of this thesis rests with the author. Access is subject to the above licence, if given. If no licence is specified above, original content in this thesis is licensed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC-ND 4.0) Licence (<https://creativecommons.org/licenses/by-nc-nd/4.0/>). Any third-party copyright material present remains the property of its respective owner(s) and is licensed under its existing terms.

### Take down policy

If you consider content within Bath's Research Portal to be in breach of UK law, please contact: [openaccess@bath.ac.uk](mailto:openaccess@bath.ac.uk) with the details. Your claim will be investigated and, where appropriate, the item will be removed from public view as soon as possible.

Can we improve how we diagnose osteomyelitis  
in the diabetic foot?

Kim Harman

A thesis submitted in part fulfilment for the award  
of Professional Doctorate in Health

School of Health

University of Bath

March 2010

COPYRIGHT

Attention is drawn to the fact that copyright of this thesis rests with its author. This copy of the thesis has been supplied on condition that anyone who consults it is understood to recognise that its copyright rests with its author and that no quotation from the thesis and no information derived from it may be published without the prior written consent of the author.

This thesis may be made available for consultation within the University Library and may be photocopied or lent to other libraries for the purposes of consultation.

I, Kim Harman, confirm that this dissertation and the work presented in it are my own achievement.

1. Where I have consulted the published work of others this is clearly attributed.
2. Where I have quoted from the work of others the source is always given. With the exception of such quotations this dissertation is entirely my own work.
3. I have acknowledged all main sources of help.
4. If my research follows on from previous work or is part of a larger collaborative research project I have made clear exactly what was done by others and what I have contributed myself.
5. I have read and understand the penalties associated with plagiarism.

In submitting my dissertation, I give permission for the title of my work to be published on the School for Health's website and for an e copy of my work to be available via the University of Bath Library.

Signed

Date

Student number 059054692

## **Contents**

<b>Acknowledgements. ....</b>	<b>8</b>
<b>Abstract.....</b>	<b>9</b>
<b>Abbreviations.....</b>	<b>10</b>
<b>Glossary of terms .....</b>	<b>11</b>
<b>Chapter 1 Background .....</b>	<b>14</b>
1.1 Definition of diabetes .....	14
1.2 The health impact of diabetes .....	15
1.3 Diabetic foot disease .....	16
1.4 Pathways to ulceration.....	20
1.5 Factors affecting ulcer development and healing capacity.....	21
Figure 1.1 Pathways to foot ulceration in the diabetic patient .....	22
1.6 Amputations in diabetes - a brief summary.....	26
<b>Chapter 2 Introduction.....</b>	<b>29</b>
2.1 Literature review search strategy .....	29
2.1.1 Review of haematological markers; .....	29
2.1.2 Review of imaging; .....	30
2.1.3 Review of ulcer classification system; .....	30
2.2 Defining osteomyelitis .....	30
2.3 Osteomyelitis as a specific complication in diabetes .....	33
2.4 Diagnosing osteomyelitis .....	37
2.4.1 Gold standard diagnosis.....	37
2.4.2 Other tests used clinically .....	39
2.4.3 Imaging techniques in the diagnosis of osteomyelitis .....	40
2.4.4 Reference test for diagnosis .....	43
2.5 The immune response.....	44
2.6 The acute phase reaction.....	45
2.7 Inflammatory blood markers; non specific.....	46
2.7.1 Neutrophil count.....	48
2.7.2 Haemoglobin .....	50
2.7.4 Erythrocyte Sedimentation Rate.....	56
2.7.5 Plasma Viscosity .....	57

2.7.6 Procalcitonin .....	59
2.8 Proposed studies using inflammatory blood markers .....	63
2.9 Proposed studies using SPECT/CT .....	65
2.10 The study and wound classification .....	65
2.10.1 Ulcer classification systems .....	65

### **Chapter 3 Methodology The use of inflammatory blood markers in the diagnosis of osteomyelitis..... 70**

3.1 Research question.....	70
3.2 Aim.....	70
3.3 Research objectives .....	70
3.4 Protocol.....	71
3.5 Summary.....	72
3.6 Keywords.....	72
3.7 Confounding factors.....	72
3.7.1 Recognition of confounding factors .....	73
3.7.2 Managing confounding factors .....	73
3.8 Multiple versus single markers .....	74
3.9 Setting of research studies .....	74
3.10 Sample .....	75
3.11 Method; Ethical statement .....	75
3.12 Method; Ethical considerations.....	76
3.13 Inclusion / exclusion criteria.....	77
3.14 Consent .....	77
3.15 Method; Wound assessment .....	78
3.16 Method; venepuncture .....	80
3.17 Data collection tool .....	82
3.18 Method; Imaging studies .....	82
3.19 Analysis; of the blood markers .....	82
3.20 Measurement of the specific inflammatory blood markers .....	83
3.20.1 Haemoglobin and Neutrophil Count.....	83
3.20.2 Plasma Viscosity .....	83
3.20.3 C Reactive Protein.....	83
3.20.4 Procalcitonin .....	84

### **Chapter 4 Results The use of inflammatory blood markers in the diagnosis of osteomyelitis ..... 85**

4.1 Statistical analysis of results of blood samples.....	85
4.2 Sample characteristics - age.....	86
Table 4.1 sample characteristics - gender .....	86
Table 4.2 sample characteristics - type of diabetes.....	86
Table 4.3 sample characteristics – diabetes treatment .....	86
4.3 HbA1c levels.....	86

Table 4.4 sample characteristics – duration since diagnosis .....	86
Table 4.5 numbers of wound types .....	87
Table 4.6 descriptive statistics in all wound types for each inflammatory marker .....	87
4.3 Summary statistics for each marker in each state separately .....	87
Table 4.7 summary statistics of each inflammatory marker in each wound condition .....	88
Figure 4.4.1 Comparison of Neutrophil Count in all wound conditions .....	89
Figure 4.4.2 Comparison of Haemoglobin level in all wound conditions (excludes renal impairment) .....	90
Figure 4.4.3 Comparison of CRP level in all wound condition .....	90
Figure 4.4.5 Comparison of Procalcitonin level in all wound conditions .....	91
4.5 Receiver Operator Characteristic Curves .....	91
Figure 4. 5.1 Receiver Operator Characteristic Curve – clean wounds .....	92
Figure 4.5.2 Receiver Operator Characteristic Curve – cutaneous infection in wounds .....	93
Figure 4.5.3 Receiver Operator Characteristic Curve – suspected osteomyelitis infected wounds .....	94
Figure 4.5.4 Receiver Operator Characteristic Curve – confirmed osteomyelitis infected wounds .....	95
4.6 Logistic Regression analysis without recognising confounders .....	96
Table 4.8 Logistic Regression analysis without recognising potential confounders predicting all wound types .....	96
4.7 Logistic Regression analysis with recognised potential confounders .....	96
Table 4.9 Potential confounders in regression analysis predicting clean wounds .....	97
Table 4.10 Potential confounders in regression analysis predicting wounds with cutaneous infection .....	97
Table 4.11 Potential confounders in regression analysis predicting wounds with suspected osteomyelitis .....	98
Table 4.12 Potential confounders in regression analysis predicting wounds with confirmed osteomyelitis .....	98
Table 4.13 Comparison of C Reactive Protein in all individuals with type 2 diabetes to those with type 2 diabetes AND on Metformin and BMI >25 kg / m <sup>2</sup> for all wound types .....	99
Table 4.14 Correlations between inflammatory markers in clean wounds .....	99
Table 4.15 Correlations between inflammatory markers in wounds with cutaneous infection .....	100
Table 4.16 Correlations between inflammatory markers in suspected osteomyelitis .....	100
Table 4.17 Correlations between inflammatory markers in confirmed osteomyelitis .....	101

## **Chapter 5 Discussion The use of inflammatory blood markers in the diagnosis of osteomyelitis .....**

5.1 Sample characteristics .....	102
----------------------------------	-----

5.2.1 Neutrophil Count.....	104
5.2.2 Haemoglobin.....	104
5.2.3 C Reactive Protein.....	104
5.2.4 Plasma Viscosity.....	104
5.2.5 Procalcitonin.....	105
5.3 Inflammatory blood markers in clean wounds.....	105
5.4 Inflammatory blood markers in cutaneous infection.....	105
5.5 Inflammatory blood markers in suspected osteomyelitis.....	107
5.6 Inflammatory blood markers in proven osteomyelitis.....	107
5.7 Receiver Operator Characteristic curve in clean wounds.....	107
5.8 Receiver Operator Characteristic curve in cutaneous infection.....	107
5.9 Receiver Operator Characteristic curve in suspected osteomyelitis.....	108
5.10 Receiver Operator Characteristic curve in proven osteomyelitis.....	108
5.11 Regression models with no confounders in clean wounds.....	108
5.12 Regression models with no confounders in cutaneous infection.....	109
5.13 Regression models with no confounders in suspected osteomyelitis.....	109
5.14 Regression models with no confounders in proven osteomyelitis.....	109
5.15 Regression models with confounders in clean wounds.....	109
5.15.1 No antibiotic use.....	109
5.15.2 Use of Insulin.....	110
5.15.3 Use of Metformin.....	110
5.15.4 Predominantly neuropathic wounds.....	110
5.16 Regression models with confounders in cutaneous infection.....	111
5.16.1 No antibiotic use.....	111
5.16.2 Use of Insulin.....	111
5.16.3 Use of Metformin.....	112
5.16.4 Predominantly neuropathic wounds.....	112
5.17 Regression models with confounders in suspected osteomyelitis.....	113
5.17.1 No antibiotic use.....	113
5.17.2 Use of Insulin.....	113
5.17.3 Use of Metformin.....	114
5.17.4 Predominantly neuropathic wounds.....	114
5.18 Regression models with confounders in proven osteomyelitis.....	114
5.18.1 No antibiotic use.....	114
5.18.2 Use of Insulin.....	115
5.18.3 Use of Metformin.....	115
5.18.4 Predominantly neuropathic wounds.....	115
5.19 Comparative results to other studies for Neutrophil Count.....	115
5.20 Comparative results to other studies for Haemoglobin.....	116
5.21 Comparative results to other studies for C Reactive Protein.....	117
5.22 Comparative results to other studies for Plasma Viscosity.....	119
5.23 Comparative results to other studies for Procalcitonin.....	120
5.24 Comparative results for other confounders.....	121
5.24.1 Metformin use.....	121
5.24.2 Antibiotic use.....	122
5.25 Correlation effect of inflammatory markers with no confounders.....	123
5.25.1 Clean wounds.....	123

5.25.2 Cutaneous infection .....	124
5.25.3 Suspected osteomyelitis .....	124
5.25.4 Confirmed osteomyelitis .....	125
5.26 Correlation effect of inflammatory markers with confounders .....	125
5.26.1 Clean wounds .....	125
5.26.2 Cutaneous infection .....	125
5.26.3 Suspected osteomyelitis .....	126
5.26.4 Confirmed osteomyelitis .....	126
5.27.1 Correlation and the marker of Neutrophil Count .....	126
5.27.2 Correlation and the marker of Haemoglobin .....	127
5.27.3 Correlation and the marker of C Reactive Protein .....	127
5.27.4 Correlation and the marker of Plasma Viscosity.....	127
5.27.5 Correlation and the marker of Procalcitonin.....	127

## **Chapter 6 Methodology The use of SPECT/CT in the diagnosis of osteomyelitis .....**

**128**

6.1 Research question.....	128
6.2 Research aim.....	128
6.3 Research objective .....	128
6.4 Protocol.....	128
6.5 Summary.....	129
6.6 Keywords.....	129
6.7 Rationale of method .....	129
6.7.1 Imaging techniques used.....	130
6.8 Imaging and the disease of diabetes.....	130
6.9 Confounding factors.....	131
6.9.1 Recognition and Managing of confounding factors.....	131
6.10 Sample .....	132
6.11 Method; Ethical statement .....	133
6.12 Method; Ethical considerations.....	133
6.13 Inclusion / exclusion criteria.....	134
6.14 Consent .....	135
6.15 Method of study .....	136
6.15.1 Using MRI as the reference test .....	136
6.16 Methodology .....	137
6.16.1 Magnetic Resonance Image acquisition protocol.....	138
6.16.2 Single Photon Computed Tomography / Computed Tomography Image acquisition protocol.....	139
6.17 Diagnosing Osteomyelitis in a MRI scan.....	140
6.18 Diagnosing Osteomyelitis in a SPECT/CT scan.....	140
6.19 Analysis of imaging results.....	141

## **Chapter 7 Results The use of SPECT/CT in the diagnosis of osteomyelitis ....**

**142**



7.1 Sample characteristics .....	142
7.2 Scan results .....	143
Table 7.2.1 Patient 1 .....	143
Table 7.2.2 Patient 2 .....	144
Table 7.2.3 Patient 3 .....	144
Table 7.2.4 Patient 4 .....	145
Table 7.2.5 Patient 5 .....	146
Table 7.2.6 Patient 6 .....	147
Table 7.2.7 Patient 7 .....	148
Table 7.2.8 Patient 8 .....	148
Table 7.2.9 Patient 9 .....	149
Table 7.2.10 Patient 10 .....	150
 <b>Chapter 8 Discussion The use of SPECT/CT in the diagnosis of osteomyelitis</b>	<b>151</b>
 <b>Chapter 9 General Discussion, Recommendations and Conclusions .....</b>	<b>154</b>
 <b>Chapter 10 Appendices .....</b>	<b>158</b>
10.1 Ethics approval .....	158
10.2 Ethics approval for amendment .....	161
10.3 Research and Development approval .....	165
10.4 Research and Development approval following ethics amendment .....	168
10.5 Patient Information Sheet .....	170
10.6 Consent form .....	175
10.7 Data collection tool .....	177
10.8 Proposed consensus criteria for diagnosing osteomyelitis in the diabetic foot .....	180
10.9 Histograms and summary statistics of each inflammatory marker in each wound condition .....	182
Neutrophil Count in clean wounds .....	183
Haemoglobin in clean wounds (not including renal impairment) .....	183
CRP in clean wounds .....	184
Plasma Viscosity in clean wounds .....	184
Procalcitonin in clean wounds .....	185
Neutrophil Count in cutaneous infection .....	185
Haemoglobin in cutaneous infection (not including renal impairment) .....	186
CRP in cutaneous infection .....	186
Plasma Viscosity in cutaneous infection .....	187
Procalcitonin in cutaneous infection .....	187
Neutrophil Count in suspected osteomyelitis .....	188
Haemoglobin in suspected osteomyelitis (not including renal impairment) ....	188
CRP in suspected osteomyelitis .....	189
Plasma Viscosity in suspected osteomyelitis .....	189

Procalcitonin in suspected osteomyelitis.....	190
Neutrophil Count in proven osteomyelitis .....	190
Plasma Viscosity in proven osteomyelitis .....	192
Procalcitonin in proven osteomyelitis .....	192
10.10 Regression analysis results .....	193
10.10.1 Regression models without confounders .....	193
10.10.1.1 Regression models without confounders in clean wounds .....	193
10.10.1.2 Regression models without confounders in cutaneous infected wounds.....	193
10.10.1.3 Regression models without confounders in suspected osteomyelitis .....	194
10.10.1.4 Regression models without confounders in confirmed osteomyelitis .....	194
10.10.2 Regression models with confounders .....	195
10.10.2.1 Regression models with confounders in clean wounds .....	195
10.10.2.2 Regression models with confounders in cutaneous infected wounds.....	196
10.10.2.3 Regression models with confounders in suspected osteomyelitis ....	199
10.10.2.4 Regression models with confounders in confirmed osteomyelitis ...	200
<b>References .....</b>	<b>203</b>

## **Acknowledgements.**

I wish to thank all my family and friends for their constant help during the long process of writing this thesis, for their unfailing support and encouragement to continue and belief that it would be finished. Also to the staff within the Diabetes and Endocrine Centre, the Pathology and Imaging departments of the Royal United Hospital, Bath and Queens University, Belfast who provided clinical help, to Baileys Instruments for providing the 10 gramme monofilament for neuropathy testing and to Becton Dickinson pre-analytical diagnostics for providing the venepuncture materials.

I also wish to thank my supervisors Dr Gordon Taylor Senior Lecturer Medical Statistics of the University of Bath and Dr Tony Robinson Consultant Endocrinologist at the Royal United Hospital, Bath.

## **Abstract**

Soft tissue infection in a diabetic foot with an ulcer is often clinically obvious but the diagnosis of osteomyelitis underlying a diabetic foot ulcer is challenging. It has been calculated that there are over 1 million amputations worldwide for diabetes related complications every year, many preceded by an ulcer complicated by osteomyelitis.

This research encompasses two studies attempting to add to the ways in which osteomyelitis is diagnosed.

The first was examining the role of inflammatory blood markers in recognising and separating ulcers with cutaneous infection from both suspected and proven osteomyelitis. The response of the body to produce these markers when an injury occurs is well known but arguments exist as to the capacity of the individual with diabetes to do so. Despite the recognition and allowance for common confounding factors no trend was found. This study may have been more difficult than originally thought due to the many interactions of the diseased state of diabetes, the drugs used to control it and the many other confounders that would have influenced the inflammatory process and as such the level of the markers.

The second study was comparing a new form of scanning technique (SPECT/CT) to the technique most commonly used as a 'gold standard' – MRI. The results of each type of scan were compared to the clinical diagnosis and each other. The SPECT/CT scan appears to show some good results and may be a more suitable scan for individuals who are unable to have a MRI for example due to the need to introduce a renally excreted drug to help make the images clearer but it does mean introducing a small amount of radiation into the individual.

## **Abbreviations**

ANC – Absolute Neutrophil Count

BMI – Body Mass Index

CKD – chronic kidney disease

CRP – C Reactive Protein

CVD – cardiovascular disease

CT – computed tomography

Cutinf – cutaneous infection

Conom – confirmed osteomyelitis

eGFR – estimated Glomerular Filtration Rate

ESR – erythrocyte sedimentation rate

Hb – Haemoglobin

HbA1c – glycosylated Haemoglobin

MRI – Magnetic Resonance Image/ing

NC – Neutrophil Count

NHS – National Health Service

PCT – Primary Care Trust

PCN - Procalcitonin

PV –Plasma Viscosity

PVD – peripheral vascular disease

ROC – Receiver Operator Characteristic (of a curve)

SPECT/CT – Single Photon Computed Tomography / Computed Tomography

Susom – suspected osteomyelitis

WBC – white blood cell

## **Glossary of terms**

Anaemia; a reduction in the Haemoglobin level below what is considered to be normal. The precise level to be considered pathological in this study is that proposed by the National Kidney Foundation (2001) of 11 g/ dl irrespective of gender.

Body Mass Index; is determined by a person's height and weight. Because the BMI calculation requires only height and weight, it is inexpensive and easy to use. The Metric System uses the formula weight in kilograms divided by height in meters squared (weight (kg) / height (m)<sup>2</sup>).

C Reactive Protein; an acute phase inflammatory mediator which is an immunoglobulin which in an antigen complex triggers the complement cascade. It has the property of being able to bind to a polysaccharide in the cell wall of pneumococci bacteria, the fraction of the wall being fraction 'C'. The bacterial polysaccharides and phospholipids released by the damaged tissue also become activators of the Complement pathway. At the cellular level C Reactive Protein initiates opsinisation and phagocytosis of invading cells. It also binds to and detoxifies endogenous toxic substances produced as a result of tissue damage. 'Normal' levels considered to be <5mg per litre.

Cardiovascular disease; determined by the prescription and use of medications including anti-hypertensives, diuretics, beta-adrenoceptor blocking drugs, nitrates, calcium-channel blocking drugs (and other antianginal drugs), anticoagulant and antifibrinolytic drugs (when clearly used for cardiac disease), and lipid regulating drugs.

Charcot (neuro-arthropathy); a non-infectious destruction of bone and joint found in individuals with neuropathy.

Chronic Kidney Disease; a decline in renal function noted to have been occurring over a period of time over the normal expected age related decline of <1ml/min/year.

estimated Glomerular Filtration Rate (eGFR); Estimated using the Modification of Diet in Renal Disease (MDRD) method. The formula for this method uses serum creatinine, age and gender to determine renal function. It is recognised this may under estimate the extent of kidney damage present (Chudleigh, Dunseath, Peter *et al* 2008) and no account was taken of the confounding factor of obesity. The bias of the MDRD increases with increasing body weight (*ibid*) which may be significant in type 2 diabetes. The same method was however used throughout the study.

HbA1c; In the haemoglobin of the erythrocytes glucose sticks to the haemoglobin to make a 'glycosylated haemoglobin' molecule, called haemoglobin A1C or HbA1C. The more glucose in the blood, the more haemoglobin A1C or HbA1C will be present in the blood. As erythrocytes live for 8 -12 weeks before they are replaced by measuring the HbA1C it can produce a mean blood glucose over the last 8-12 weeks. A normal non-diabetic HbA1C is 3.5-5.5%. In diabetes 6.5% indicates 'good' metabolic control.

Neuropathy; unable to detect a 10g monofilament in at least three sites from a total of ten tested (following the work of Leese *et al* 2007 and Leese 2007).

Neutrophil Count; the number of neutrophils in a blood sample. One that is considered to be 'normal', that is without any pathology present to either cause either depression or elevation of the level, in venous blood is in this research defined as  $2 - 7.5 \times 10^9$  per litre.

Peripheral Vascular Disease (PVD); the absence of one foot pulse in the affected foot either by palpation by hand or using a hand held Döppler.

Plasma Viscosity; a measure of the proteins in the blood. An increase occurs in the inflammatory reaction. The increase reduces the viscosity. 'Normal' in this series is between 1.5 and 1.72 mpas.

Procalcitonin; under normal metabolic conditions is produced and secreted by the C cells of the thyroid. In severe bacterial infections and sepsis the origin is extrathyroidal. In marked inflammatory conditions the principal source is 'nonneuroendocrine parenchymal cells' e.g. lung, liver, kidney, fat, muscle and stomach. The normal level is very low  $< 0.5$  ng/ml. The level measured in the assay method used was 0.005 – 25 ng / ml.

Reduced renal function; a reduction from the laboratory 'normal' level of 90ml/min/1.73m<sup>2</sup> for an estimated Glomerular Filtration Rate (eGFR) on at least 3 occasions within the last 6 months prior to the clinic visit at which consent was taken.

Renal anaemia; the anaemia associated with reduced renal function, in this study it was assumed to be present when the estimated Glomerular Filtration Rate (eGFR) was below 60ml/min in the six months preceding consent.



## **Chapter 1 Background**

This chapter will provide an explanation of the conditions that are both causative and contributory to the diabetic foot syndrome. It will attempt to explain why the problem of diabetic foot disease is so complex and why the prevention and treatment of ulcers, and in particular the complication of osteomyelitis, is so problematic.

### **1.1 Definition of diabetes**

‘Diabetes is an illness that is life long and chronic. It is generally not acute, it is without a treatment that facilitates cure, but can be controlled. As a disease it is not painful and is not seen initially as life threatening, particularly in those with type 2 diabetes’ (Fox and MacKinnon 2002). Diabetes mellitus was defined by the World Health Organisation in 1999 in the document ‘Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications’ (World Health Organisation 1999) as ‘a chronic disease, in which inherited and/or acquired Insulin deficiency occurs. i.e. the pancreas does not produce enough Insulin or when the body cannot effectively use the Insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycaemia). Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves’. In addition to this clarity was provided about types of diabetes. This reduced the confusion caused by the various classifications. Prior to this definition, different descriptions of the various forms of the disease existed, using age or treatment type rather than pathology as a means of classification. This can be seen when seeing Insulin Dependent, juvenile onset or type 1 diabetes as one form and Non-Insulin Dependent, maturity onset or type 2 diabetes as another. This confusion was particularly the case when elderly people with type 2 diabetes became Insulin requiring – a stage of the disease process. Type 2 never becomes type 1. The accepted classification is now using the

nomenclature type 1 and type 2. In Britain Diabetes UK, the largest United Kingdom charity involved in diabetes care for both health care professionals and people with diabetes, endorsed the changes in this document. This revision of the classification of the disease came about by following the clinical stages of the disease regardless of aetiology (as this may be unknown).

## 1.2 The health impact of diabetes

It is suggested that the world is facing an epidemic of type 2 diabetes (Wild *et al* 2004). The worldwide prevalence of diabetes now exceeds 200 million, and is predicted to rise to more than 360 million in the next 20 years (*ibid*). Diabetes UK (2006) has used a model called the ‘Diabetes Population Prevalence model’ to estimate that in 2010 5% of the population of England will have diabetes. The Yorkshire and Humber Public Health Observatory estimate this to be some 2091783 people in the United Kingdom (types 1 and 2 combined) (YHPHO 2009). Shaw *et al* (2010) combining prevalence studies suggest in the United Kingdom this figure would be 2140000 for the age group 20-79 years. Using the Diamond diabetes database as a proxy for a register as none exists locally this gives 22670 from a population of some 500000 in the catchment area of the hospital trust – 4.5%.

The complications associated with long-term hyperglycaemia are many. Diabetes is a multi-system, multi-organ disease. In the lower limb the major complications relate to impaired circulatory capacity due to athero and arterio sclerosis and damage to the nervous system. These are discussed in more detail later.

Diabetes, in developed countries, is a leading cause of death and contributes enormously to disability and reduced life expectancy (Amos, McGarty and Zimmet 1997). This morbidity clearly has an enormous impact on healthcare resources (Mason *et al* 1999a). Foot ulceration is believed to occur in some 15% of the diabetes population during their lifetime (NHS Centre for Reviews and Dissemination 1999). Singh, Armstrong and Lipsky (2005) with updated figures advocate the figure could

be as high as 25%. In fact diabetic foot ulceration has a considerable morbidity of itself (Mason *et al* 1999b). They, again, suggested ulceration is found in some 5% of all people with type 2 diabetes. Currie *et al* in 1996 produced figures illustrating diabetes is responsible for 5.5% of all hospital admissions, 6.4% of out patient attendance and 9.4% bed-days in the British National Health Service. Foot osteomyelitis specifically is also recognised as an expensive and morbid disease (Henke *et al* 2005). Gordois *et al* (2003) estimated foot ulceration and amputations associated with diabetes consumed 5% of the National Health expenditure in 2001 (some £3 billion). The National Health Service National Diabetes Support Team has suggested the financial burden of diabetic foot disease in 2008 (for the United Kingdom alone) was in the order of some £252 million per annum.

A significant number of patients with diabetes will have multiple hospital admissions following surgery because of further episodes of foot ulceration and further amputations (Schofield *et al* 2006). The survival for those who develop foot disease resulting in amputation is very poor with a two year mortality of up to 50% (Ragnarson and Apelqvist 2004, Tentolouris *et al* 2004 and Schofield *et al* 2006).

### 1.3 Diabetic foot disease

While the spectrum of diabetes-related foot disease comprises chronic ulceration (with or without associated infection of soft tissue and / or bone), critical ischaemia and acute Charcot foot, most attention is focused on the management of the chronic, non-healing ulcer (Jeffcoate *et al* 2008). The health burden of diabetic foot disease is huge and has been recognised as an economic burden for both patients and their families (Boulton *et al* 2005) and as the most costly societal part of diabetes care (Reiber *et al* 1998, NHS Centre for Reviews and Dissemination 1999). More hospital beds are occupied by patients with diabetes associated foot problems than all of the other complications combined (Elkeles and Wolfe 1991). This is despite diabetes itself being the leading cause of both renal failure and also blindness in the working

age population. Patients with diabetes have a ten fold greater risk of soft tissue infection and bone infection in the lower extremity compared with healthy individuals (Singh, Armstrong and Lipsky 2005, Lavery *et al* 2006, Dinh, Abad and Safdar 2008). The need for hospitalisation in foot disease often heralds the need for amputations (Dinh, Abad and Safdar 2008). Foot ulceration is recognised to be associated with frequent visits to health care professionals (Lipsky *et al* 2004). It is estimated some 20% of patients with diabetes with a foot infection are hospitalized at some point (Crolle *et al* 1996, Chatha *et al* 2005). In their large study of 1666 consecutive patients Lavery, Armstrong and Wunderlich (2006) saw the development of a foot infection increases the risk of hospitalisation 55.7 times (95% CI 30.3 -102.2  $p<0.001$ ) and the risk of amputation 154.5 times (95% CI 58.5 – 468.5  $p < 0.001$ ). It has been suggested a diabetic foot problem increases the risk of non-traumatic amputation to 15 times that of the non-diabetic population (Krentz and Bailey 2001). Crolle *et al* (1996) estimate diabetes accounts for 80% of ‘infectious amputations’, Lavery *et al* (2006) place this nearer 90%. It has been calculated that there are over 1 million amputations worldwide for diabetes related complications every year (Bakker *et al* 2005) which equates to someone, somewhere, losing a leg because of diabetes every 30 seconds of every day (*ibid*). The data available locally for the numbers admitted for lower extremity amputations using Hospital Episode Statistics was in 2003 (latest figures available) an age standardised ratio (in the general population) in Avon, Gloucestershire and Wiltshire Strategic Health Authority was 4 per 100000, Bath and North East Somerset Primary Care Trust being lower at 2 per 100000 (Ho 2006). The data on both incidence and prevalence of osteomyelitis is not kept in part due to the difficulty in accurate diagnosis of the condition. Despite advances in the recognition of osteomyelitis the, now old, study by Gürlek *et al* (1998) shows an odds ratio of osteomyelitis being a predictor of amputation with an odds ratio of 3.73 (95% CI 1.08 – 12.6  $p=0.04$ ). Lipsky (2008) describes the development of osteomyelitis of the foot being a ‘potentially catastrophic event for a person with diabetes’.

Although now long standing, the work of world-renowned diabetes physician Jan Apelqvist and his team in 1994 suggested costs associated with ulceration and non-

traumatic amputation range from 43000 –65000 US dollars (1990 currency rates) per person per amputation. These figures are more useful than many of the preceding ones as it includes the costs that are necessary after amputations including rehabilitation and care provided in primary care. The range recognises the cost differences of minor and major surgery with the total cost to healing. The Eurodiale study (Prompers, Huijberts, Schaper *et al* 2008) has updated this costing in a well constructed study using prospective data collection. In thirteen European countries data was collected and analysed over a weighted resource utilisation scheme and recognised the ‘highest costs per patient were for hospitalisation, antibiotics and amputations and other surgery’. Based upon data from 821 patients the costs compared to the work of Apelqvist *et al* (1994) the cost was € 10000 today compared to € 7412 (at 2005 exchange rates). Economic comparisons are difficult because of many differences including study design (prospective *vs.* retrospective and primary *vs.* specialist care), selection of patients, type of foot lesions, type of healthcare systems and settings, treatment practice and time for analyses. Boulton *et al* (2005) also suggest the need to clarify proportional costs for different uses of resources in relation to total costs.

Any clinician treating a patient with a diabetic foot infection should be considering several issues, key amongst these being how broad spectrum the antibiotic regimen should be and by what route (oral or intra-venous) should it be administered, when urgent surgical or other speciality consultations are required, and whether hospitalization is required (Jeffcoate and Harding 2003 and Lipsky *et al* 2004). It has been suggested the most important factor affecting these decisions is the clinical severity of the infection (Lipsky *et al* 2004 and Bakker *et al* 2005). The reasoning being made on sound evidence that poor treatment is likely to produce adverse outcomes that may ultimately mean loss of limb. Unfortunately at present clinicians have little evidence-based guidance for identifying which patients have a ‘severe’ foot infection or which clinical findings are associated with a poor outcome (Macfarlane and Jeffcoate 1997).

Infection can be divided into three categories: superficial, local soft tissue and spreading (cellulitis), and osteomyelitis (Jeffcoate and Harding 2003). The liability to infection occurring with sensory neuropathic ulceration means that extensive tissue damage, and indeed destruction can occur rapidly, possibly without the injury being apparent. Depth of wound was purported to be an accurate indicator by the Infectious Diseases Society of America's Diabetic Foot Infection Classification System (Lipsky *et al* 2004a) and this now has been validated to predict outcomes of diabetic foot infection in a well conducted longitudinal trial of 1666 patients (Lavery *et al* 2007). The results of this study show statistical significance towards risk for any amputation, including higher-level amputation and any lower extremity amputation with increasing infection severity, all at  $p < 0.001$ . In this same study the presence of infection to joint and / or bone level was noted to be in the 'moderate' to 'severe' categories with only 'mild' infection not contributing to the likelihood of the above noted outcomes.

Acute osteomyelitis is frequently seen from contiguous spread from such tissue damage (Embil 2000), becoming chronic due to the lack of sensation allowing it to pass unnoticed (*ibid*). Foot infection is perhaps the most important manifestation of diabetic foot disease owing to the potential for osteomyelitis, septic arthritis, and generalised sepsis with endotoxic shock, the latter having a 40%- 90% mortality rate (Sartoris 1994). There are also the consequences of a relatively high rate of limb amputation and poor long-term prognosis for survival being one of the most serious and costly problems associated with diabetes mellitus (Leichter *et al* 1988). Chronic osteomyelitis typically has an insidious presentation, involving necrotic or ischaemic bone and surrounding soft tissue (Lipsky 1997). Almost all diabetic patients with foot osteomyelitis have chronic contiguous infections (*ibid*). A study by Roglic and Unwin (2010) estimates using the latest WHO life tables, country specific diabetes prevalence and relative risk of death for a person with diabetes, that in 2010, in Europe, the %age of deaths, attributable to diabetes will be 11% in age group 20-79 years.

The burden of diabetic foot disease is probably set to increase in the future since the contributory factors to foot disease, such as peripheral neuropathy and vascular disease, were present in more than 10% of people at the time of diagnosis of type 2 diabetes in work which is now old (UKPDS 33 1998). The prevalence of diabetes has risen significantly since this time (see health impact).

#### 1.4 Pathways to ulceration

The specific complications that occur to make the syndrome of ‘the diabetic foot’ are peripheral neuropathy, liability to infection and a reduced peripheral arterial blood flow – ischaemia, as described by Edmonds *et al* in their seminal paper of 1986. Slovenkai (1998) adds deformity in his ‘major threats’ to the diabetic foot.

Foot wounds are amongst the most common and severe complications of diabetes (Singh, Armstrong and Lipsky 2005). This has also meant that they are responsible for the most frequent cause of diabetes associated hospitalization (*ibid*).

The neuropathy that develops can be, in part, a loss of protective sensation - this is the major factor, allowing minor damage to pass unnoticed. Other neuropathies include motor imbalance with abnormal pressures associated with it leading to ulceration (Plank, Graham and Hyer 2000) and also local sympathetic denervation, this having the result of changing the levels of hydration in the skin (Hill *et al* 1999).

The liability to infection occurring with sensory neuropathic ulceration means that extensive tissue damage and indeed destruction can occur rapidly, possibly without the injury being apparent.

The poor circulation due to accelerated arteriosclerosis and increased atheroma formation leads to

- Poor tissue perfusion (Krentz and Bailey 2001)

- A reduced inflammatory response in reaction to damage
- A reduced ability to fight infection
- A reduced ability to allow healing to occur (Hill *et al* 1999).

The neuropathy of the autonomic nerves intensifies the poor inflammatory response potentially causing it to be greatly reduced. Both neuropathy and vasculopathy are strong independent risk factors for the incidence of foot ulcers (McNeely *et al* 1995).

The interaction of these to produce the syndrome of the diabetic foot is illustrated well by the diagram in Figure 1.1 on page 22.

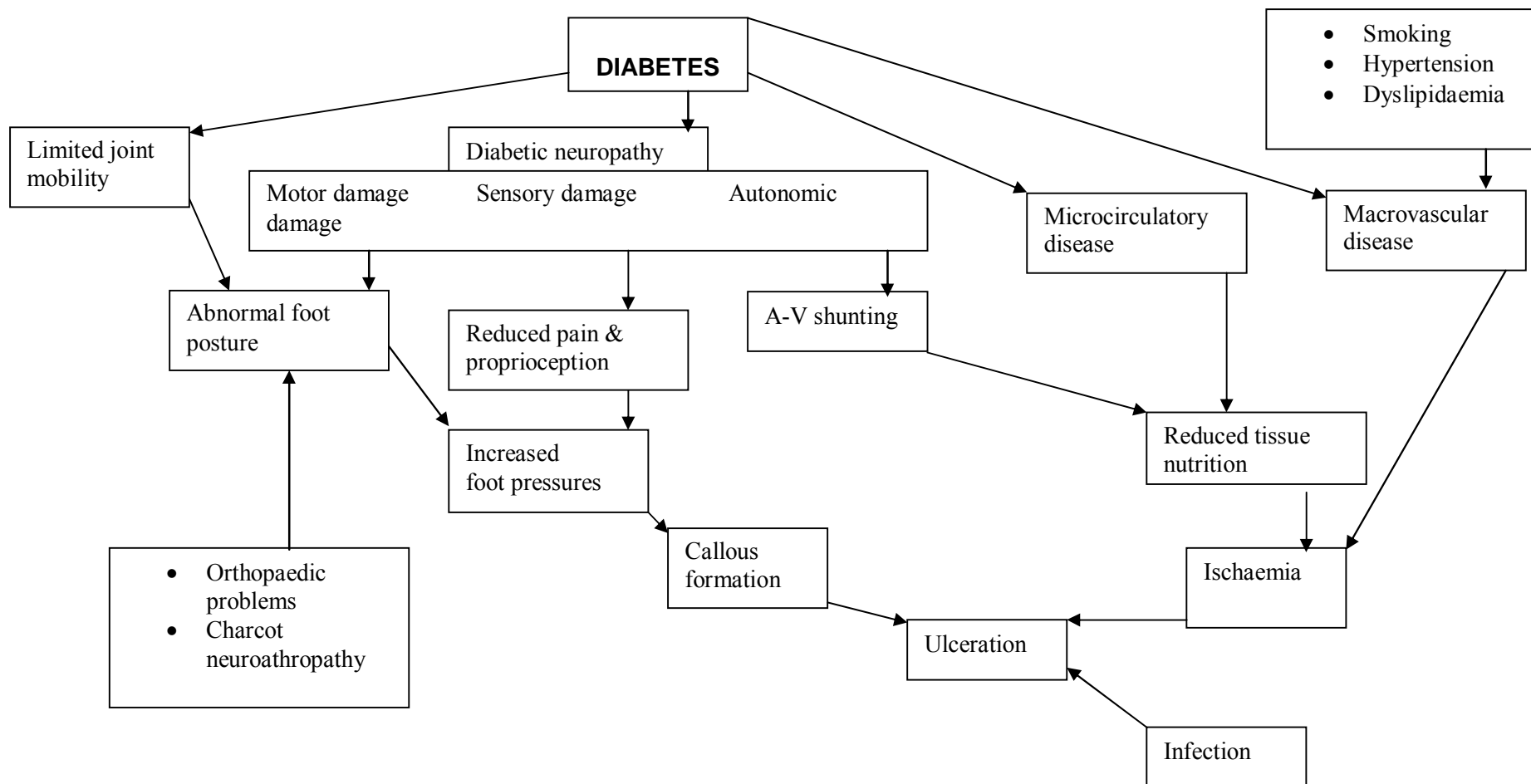
### 1.5 Factors affecting ulcer development and healing capacity

The assessment of the etiologic causes and determinants of potential to heal the diabetic foot include judgment as to the degree of nerve damage, circulatory impairment and extent of infection. There are numerous methods of estimating these aspects.

It is now well accepted that peripheral neuropathy is an important pathophysiological risk factor for developing foot ulcers (McNeely *et al* 1995). Nerve damage, or neuropathy, has several features that affect the foot. Most commonly recognised is the numbness that allows damage to go undetected. Other elements include damage to nerves supplying foot muscles. The result of this is in small muscle paralysis and a structurally altered foot shape – becoming more prone to trauma and autonomic nerve damage. The poor autonomic functioning produces a foot with a poor inflammatory response to injury and a poor recovery from cold exposure both of which can allow unsuspected tissue damage to occur. The loss of pain sensation is usually clinically assessed by the use of a monofilament, a tuning fork or by the application of a



Figure 1.1 Pathways to foot ulceration in the diabetic patient  
(from Williams and Pickup 1999)



controlled electrical voltage using an instrument called a biothesiometer (or neurothesiometer). The latter two measuring response to vibration.

These testing mechanisms can all be criticised for being evaluations of subjective attributes of sensations evoked by natural stimulation of cutaneous stimulation (American Diabetes Association and the American Academy of Neurology 1988). Diagnostic tests should fulfil the following criteria: validation (presence of an independent reference standard, adequate spectrum and number of patients, standardization, and soundly based selection), predictive value, manageability (reproducibility, performance in clinical practice) and hierarchy (implying that patients with the same score have difficulties or problems with the same items) (Meijer *et al* 2000). It should be recognised that the clinical assessment should be tailored to the type of study, rapid, relatively simple measures may at times be appropriate as in this instance (American Diabetes Association and the American Academy of Neurology 1988).

At present there is no international agreement on either the anatomic sites or number of sites to be tested using monofilaments (Forouzandeh *et al* 2005). Nor is there agreement about what constitutes a significant loss of sensation to this testing method (*ibid*). Without consistent, reliable, measurements when testing sensation any form of assessment will not produce meaningful results (Mawdsley *et al* 2004). The sensitivity and specificity of tuning forks (used at the hallux) and monofilaments (used at eight sites) appear from the work of Miranda-Palma *et al* (2005) to be similar. Their research does appear to have followed the typical practice of use of the instruments and as such is practically useful in a clinical setting.

In work comparing a tuning fork to a Neurothesiometer, the tuning fork was found to be unreliable (O'Neill, McCann, Lagan 2006). The authors of this paper admit to the sample being very small (n=21) and accept with it being potentially biased based upon only one testing site with an overall agreement of the tools of 66.2%. Biothesiometry is one of few quantitative measures of neuropathy suitable for use in a busy clinical environment (Cassella, Ashford and Kavanagh-Sharp 2000).

Of the screening methods the most reliable, valid and simple to use the monofilament emerges as the method of choice (McNeely *et al* 1995, Meijer *et al* 2000 and Meijer *et al* 2002). Olmos, Cataland, O'Dorisimo (1995) and Burton (2001) include cheapness in their list of positive attributes to the monofilament. The principle behind the use of the 10g monofilament is simple. It is calibrated to buckle when a force of 10g is exerted, if the patient cannot feel the pressure the foot is considered to be neuropathic (Olmos *et al* 1995). The standardization of length and thickness of the filament is such that they buckle at reproducible forces. This means the amount of pressure administered when applied to the skin is more a function of the instrument rather than of the examiner (*ibid*) – this is clearly very important. However it is difficult to interpret the results of some of the papers to determine the methods and determination of the results. This is because in part to the inability to ensure the research subjects have understood the procedure to be undertaken (Holewski *et al* 1998) and that clinicians have undertaken a standardised methodology (*ibid* and Thomson *et al* 2001). Different methodologies may give dissimilar results and make it difficult to compare the data between clinicians and centres (*ibid*). After reading the work by Holewski *et al* (1998) and Burton (2001) the assessment procedure that was determined to be the most effective was the use of a monofilament on all of the metatarsal heads and also the plantar surface of the hallux with pathological loss of sensation being an inability to feel three, or more, of the six sites, tested. This is not dissimilar to the suggestion by McGill *et al* (1999) of the loss being determined by the loss of recognition at the first and fifth metatarsal heads.

Diabetes is the most powerful risk factor for peripheral arterial disease (Stuart *et al* 2004). The unreliable nature of signs and symptoms of lower limb arterial insufficiency in diabetes means non invasive tests are essential to achieve effective screening (Fagila *et al* 1998).

Assessment of arterial circulation, in the absence of the technically difficult and not easily available to all, (colour duplex scan and digital pulse pressures,) uses simple palpation or use of a döppler to determine presence or absence of pulses. This has been shown to be adequate for screening (Leese *et al* 2007).

The simple palpation of pedal pulses has been assessed, against other screening methods, and found to be ‘highly sensitive .. in individuals with .. diabetes’ (Williams, Harding and Price 2005). However of note is the fact that the same authors report a reduced sensitivity and poor specificity of using pulse palpation when associated with neuropathy. Other factors have been noted to make the test subjective including ambient room temperature (Williams, Price and Harding 2003.)

The evaluation of circulation can be seen to include skin changes characteristic of a ‘foot at risk’ (Springett and White 2002). These changes are indicative of the potential for slow, or no, healing (*ibid*). The measurement of an Ankle: Brachial Pressure Index has not been found to be reliable (De Graaf *et al* 2001, Weatherley *et al* 2006). This was both using a döppler probe and DINAMAP™ blood pressure monitor. An Ankle: Brachial Pressure Index is a ratio determined by the comparison of systolic arterial blood pressure in the arm to the foot; it should ideally be 1 i.e. the same in both vessels. Diabetes associated calcification of the vessel will elevate it as the calcified lower limb vessel will not be able to be compressed in reading the blood pressure making the value above 1, poor peripheral blood flow can reduce it below 1.

Overall Leese *et al* (2006 and 2007) have demonstrated that using palpation of pedal pulses and using a monofilament to assess neuropathy can detect individuals likely to

be at 'low, moderate or high risk of ulceration' and predict capacity to heal or not. The tool they developed, and validated uses 'simple' pedal pulse palpation. The simplicity of this validated assessment method means it was adopted in this study.

The recognition of the measures of sensory neuropathy being imperfect means any classification system such as that proposed by Leese *et al* (2006) and the S(AD) SAD developed by Macfarlane and Jeffcoate (1999) are qualitative and have a descriptive taxonomy. Some of the different ulcer classification systems have been evaluated and found to be useful for clinical classification, audit or research – never all.

From the above it can be seen that the diabetic foot is a complex collection of disease processes that interact to cause further complications and that the recognition of pathology can itself be problematic.

The risk for bone infection is recognised from a study by a well respected group of physicians interested in the diabetic foot as almost seven fold higher among patients in whom the wound penetrates to underlying bone (Lavery *et al* 2006). Despite the best efforts of even experienced clinicians many persons with osteomyelitis require a lower extremity amputation (Lavery *et al* 2008). Recognition of the severity of infection remains difficult. This study was developed in the anticipation that the use of simple blood tests may assist the early recognition of osteomyelitis.

### 1.6 Amputations in diabetes - a brief summary

It has been suggested by Vamos *et al* (2010) the most costly and devastating end point of diabetic foot problems is lower extremity amputation. The clinical prognosis for these individuals is very poor. Kald, Carlsson and Nilsson (1989), Reiber, Boyko and Smith (1995), and Toursarkissan *et al* (2002) all recognising between 9% and 20% requiring a further amputation within twelve months and between 28% and 51% within five years.

In a prospective study of amputations Nather *et al* (2008) found 95% had diabetes and stepwise logistical regression analysis indicated the significance of both peripheral vascular disease and infection. Another study by Fosse *et al* (2009) shows a crude rate, not including traumatic etiologies, of 349/100 000 of amputations in people with diabetes. This makes it twelve times higher in people with diabetes compared to not having diabetes. These later figures were produced using a nationally based hospital discharge database recording major diagnoses and procedures based on International Classification of Diseases (ICD) version 10 coding. The acknowledgement that diabetes per se is not provided with a specific cost allowance may result in underreporting. Vamos *et al* (2010) using a very thorough search of ICD 10 codes with hospital episode statistics have shown an alarming rise in both major and minor amputations in type 2 diabetes. They suggest in the period of 1996-2005 minor amputations have risen from 2.4 to 4.1 per 100000 population, with major amputations rising from 2.0 to 2.7 per 100000 population. The personal and societal cost of any amputation is clearly immense in diabetes (Vamos *et al* 2010).

Osteomyelitis as an infective process is a common precursor of amputation particularly in diabetes. The other factors affecting healing in diabetes often result in amputation being used as cure where medical treatments fail. This is acknowledged by Ebsov, Schroeder and Holstein (1994), Jeffcoate and van Houtum (2004) and Congdon (2006) who all suggest that the upward trend is likely to reflect the increase in incidence and prevalence, improved survival of diagnosed individuals, greater awareness of diabetic foot disease and improved specialist services being available to individuals who may have previously died with an unhealed ulcer.

This chapter has given some background into why diabetic foot disease and in particular bone infection is so problematic and needs further investigation.

The following chapters will give further information about the literature searching strategy of the material used within this thesis, and the background to the work carried out, the nature, diagnosis and impact of osteomyelitis, the immune response

to infection, the methodologies involved in the studies performed and discussion of the results found.

## **Chapter 2 Introduction**

This chapter will establish the purpose of the studies of this thesis in relation to the work that has been performed in the area to date. It will explain the concepts of the reaction that occurs following an infection and how the studies undertaken here relate to this.

### **2.1 Literature review search strategy**

The literature was searched for material written from the 1970 to the present day for articles about diabetes foot disease and both imaging techniques and blood markers. Little work about the subject areas has been written and so a considerable time period was required to be examined. Additionally the various ulcer classification systems were considered to determine which maybe of use within the study. The databases used included CINAHL, MEDLINE, British Nursing Index, Embase and AMED. The only limit being placed being 'Humans'.

#### **2.1.1 Review of haematological markers;**

The search for the use of blood tests in the recognition, diagnosis or monitoring of osteomyelitis included using the terms 'foot ulcer', ulcer\* (as a truncation for ulcers and ulceration) and 'diagnos\*', as above, 'bone infection' and 'osteomyelitis' with the expressions of 'acute phase reaction' and 'inflammatory marker'. In addition each marker was included into this baseline search with the different terms commonly used for each marker, for example Leukocytes (and Leucocytes, or white blood cells) as a broad term and also Neutrophils specifically and both plasma and blood viscosity (and rheology). The different spellings of Haemoglobin and anaemia between the English and American medical publications was recognised and included in the search strategy.



Only one study was found to be prospective with consecutive recruitment and include both in and out patients (Newman *et al* 1991). The vast majority of papers found were found to only have studied hospital in patients and as such were of limited applicability to this study. This is a surprise as the use of an out patient setting to deliver diabetes care is not unique to the British Isles.

#### 2.1.2 Review of imaging:

The search terms included ‘imag\*’ as a truncation of image and imaging ‘diabet\*’, ‘diagnos\*’, Diabetes and diabetic being truncated to ‘diabet\*’ to include both, diagnos\* being a truncation of diagnosis and diagnostic.

#### 2.1.3 Review of ulcer classification system:

The search terms used were diabet\*, ulcer\*, both as before, and classif\* to find the classifications and then combining each of the found systems with varying terminology to determine reliability, validity and robustness.

### 2.2 Defining osteomyelitis

Bone infection in the diabetic foot is always a complication of a pre-existing infected foot wound (Hartemann-Heurtier and Senneville 2008). Despite this the diagnosis of osteomyelitis underlying a diabetic foot ulcer is challenging because of the lack of a single, non invasive, highly sensitive and specific test (Dinh, Abad and Sadfar 2008).

A continuum has been recognised by Lipsky (1997) that involves soft-tissue infection, leading to osteitis and on to osteomyelitis. As a pathophysiological sequence it is very difficult to discern where an individual wound lies, as the signs and symptoms are subtle and no different between bone and soft-tissue (*ibid*). The

recognition of bone infection is compounded by the fact that there is no consensus in defining osteomyelitis (Berendt and Lipsky 2004). As a result of this diagnosis has often been made on a combination of good clinical judgement and tailored investigations (*ibid*). To make it more confused Delcourt *et al* (2004) maintain that diagnosis is often difficult clinically but Paluska (2004) agrees with Berendt and Lipsky (2004) suggesting the diagnosis is primarily a clinical one. Treece *et al* (2004) agree stating 'because there is a relative scarcity of scientific evidence on which to base decisions management programmes are to a large extent defined by the experience and skill of the individual clinician, as well as their preconceptions, available resources and the conventions and constraints of local health care'. Although clinical judgement may be misleading in the low grade infections due to lack of clinical manifestations (Newman *et al* 1991) it is proposed as a measure to be used in the initial classification of the ulcers in this study followed by imaging where appropriate (see later).

Using the clinical 'probe to bone' test, or visualising bone, will be used as an inclusion criterion is justified as this simple clinical test has a high specificity (Newman 1991, Grayson *et al* 1995, Crolle *et al* 1996, Chatha *et al* 2005). Jeffcoate and Lipsky (2004) are bold enough to categorically state 'bone that is visible at the base of an ulcer is likely to be infected'. The author regularly uses this in clinical examination, with other factors if osteomyelitis is suspected. Grayson *et al* (1995) also recognised 66% sensitivity and a positive predictive value of 89% for this simple clinical test. Kaleta, Fleischli and Reilly (2001) used this investigation as a diagnostic test in their study of osteomyelitis. Arguments have been made against the claimed sensitivity and predictive values of this test on the grounds of pre-test probability (Shone *et al* 2006). The result of these latter researchers suggested a prevalence of 20.2% of ulcers are complicated by osteomyelitis and was based on clinical signs of infection. Both reports give a high prevalence rate but it is important to recognise that they were both conducted in specialist clinics where the rate may be expected to be higher. Even more lately Lavery *et al* (2007) have suggested,

comparing the probe to bone test to their gold standard of positive bone biopsy culture, which produced a positive predictive value of between 57% and 62%. A possible criticism of the original research is that it was based on in-patients that were in hospital because of infection; a possible criticism of the later work by Shone *et al* (2006) is that it only involved 81 patients. Only in the paper by Lavery *et al* (2007) is a definition of a positive test given as ‘palpating a hard or gritty substance’. They also raise the problems associated with inter-rater and intra-rater reliability that has yet to be evaluated in any study. However the available evidence to date does not justify using any one, or combination of, clinical findings as a diagnostic criterion (Jeffcoate and Lipsky 2004).

In addition to the lack of definition of what the disease process actually is there is no clear agreement as to when the process can be classified as acute or chronic. The stage of osteomyelitis is defined as being related to the clinical course, or histological findings, or duration by Paluska (2004). There is some agreement between Mandell (1996), Jones, Anderson and Stiles (1997), Lew and Waldvogel (1999), Cunha (2002) and Paluska (2004) when they refer to chronicity relating to time – specifically over 10 days according to Lew and Waldvogel. ‘A continuous low grade’ is a definition used by Mandell (1996). Haas and McAndrew (1996) state chronic osteomyelitis is defined by its ‘refractoriness to cure by antimicrobials alone’.

A scheme for the diagnosis of diabetic foot osteomyelitis for research purposes was reported by Berendt *et al* in 2008 (see Appendix 8). This was in an attempt to ensure that comparisons of outcomes at different medical centres could be compared. The report was from a consensus from the International Working Group on the Diabetic Foot and consisted of extensive literature searching and expert opinion. The paper reporting the suggested scheme acknowledges that a consensus diagnostic scheme for research requires greater specificity than clinical practice that requires sensitivity.

Additionally it is recognised that where no single criterion is sufficiently reliable to make an absolute diagnosis the results of a range of clinical, laboratory and imaging findings is likely to be beneficial. This is where this reported research may add to the body of knowledge about the use of laboratory data.

The sequence of events when infection in a diabetic foot spreads can be rapid, occurring in days or even hours, especially in an ischaemic limb (Lipsky *et al* 2004). This is illustrating again the need for accurate diagnosis. The role foot ulceration has in association with pedal osteomyelitis is graphically illustrated by Bamberger, Daus and Gerding (1987) when they claim 94% of bone infection is directly associated with ulceration. Infection plays a major role in healing impairment, hospitalization and limb loss (Jeffcoate and Harding 2003). Dealing with osteomyelitis has been suggested to be the most difficult and controversial aspect in the management of diabetic foot infections recognised by all Lipsky (1997), Embil (2000), Snyder *et al* (2000), Snyder (2000) Lipsky *et al* (2004) citing Eckman *et al* (1995), and Jeffcoate and Lipsky (2004) to emphasise this point. This also reinforces the importance of early and accurate diagnosis.

### 2.3 Osteomyelitis as a specific complication in diabetes

The lifetime risk of developing a foot ulcer, the most common precursor to osteomyelitis, is about 25% for individuals with diabetes (Singh, Armstrong and Lipsky 2005). A well conducted study using a large number of patients in a prospective longitudinal study and including out patients by Lavery *et al* (2006) indicates over half develop some form of infection and some 20% develop bone culture - proven osteomyelitis. Although soft tissue infection in a diabetic foot with an ulcer is often clinically obvious, the diagnosis of osteomyelitis underlying a diabetic foot ulcer is often challenging (Dinh, Abad and Sadfar 2008). Osteomyelitis (bone infection) most commonly occurs in elderly patients when they have diabetes

or peripheral vascular disease (Mader, Shirtliff and Calhoun 1997, Cunha 2002). Vascular disease itself is a complication of diabetes. The true prevalence of osteomyelitis in diabetic foot ulcers is not known (Giurato and Uccioli 2006). Wrobel and Connolly (1998) and Newman *et al* (1991) suggest from complex studies it may be as high as 60%. This Newman *et al* (*ibid*) study used bone biopsy and culture that is not commonly performed in the United Kingdom. Jones, Anderson and Stiles (1987) note the 'high rate' of occurrence in people with diabetes in their series studying osteomyelitis in a general hospital. Typically bone contamination results from the spread of infection from soft tissue in an ongoing process that can take several weeks. Major pathogens adhere to bone by expressing adhesion factors for components of bone matrix (Hartmann-Heurtier and Senneville 2008). Accurate diagnosis of osteomyelitis is essential to optimise outcomes (Dinh, Abad and Sadfar 2008). It is an infection involving the periosteum, cortex and/or medullary canal (Cunha 2002). It has been estimated that osteomyelitis complicates approximately 30% of diabetic foot infections (Keidar *et al* 2005 and Jeffcoate *et al* 2008). It is clinically difficult to distinguish between infective and neuropathic lesions of bone (the latter being called either Charcot joints or neuroarthropathy) (Berendt and Lipsky 2004) and yet it is crucial as the treatment is radically different, although both often end up resulting in a lower extremity amputation (*ibid*). This is because osteopenia, bone destruction and bone repair is common to both conditions (*ibid*). Bone infection however is almost always a consequence of neuropathic skin ulceration, leading to soft tissue infection and subsequent bone involvement (Mader, Shirtliff and Calhoun 1997, Berendt and Lipsky 2004). The risk of infection is seven times higher in those whose wound penetrates to the underlying bone (Lavery *et al* 2006). Despite this work by Jeffcoate *et al* (2008) as the representatives of the International Working Group on the Diabetic Foot there are no clear protocols for the defining and treating osteomyelitis of the foot in diabetes. A progress report has been produced by Berendt *et al* (2008) using a combination of clinical, imaging and laboratory results to produce a suggestive diagnostic algorithm but this has yet to be validated. This algorithm does not include all the tests reported in this thesis investigating the use of inflammatory blood markers.

There is no clear evidence what proportion of ulcers are complicated by osteomyelitis. Estimates have been made and have been discussed earlier. A very crude estimate made by the author using doctors specifically interested in diabetes and podiatrists interested in diabetes at a national conference about diabetes gave wide ranging estimates of the number felt to have bone infection. This varied from 5% to 60% in doctors and from 2% to 40% in podiatrists. Lavery *et al* (2007) in a longitudinal cohort study found 247 of 1666 patients developed foot wounds (15%) and of these osteomyelitis was found in 30 (12%). They say that of these 30 12% had a foot wound and 20% a foot infection. It is not clear if the infection was a complication of the wound, it would be reasonable to suggest so but the researcher has experience of a Magnetic Resonance Imaging (MRI) scan suggesting bone infection in an individual with no open wound following bone surgery some months previously. Using the minimum number of individuals attending the researcher's specialist diabetes foot clinic for whom a MRI scan in 2007 was requested as a proxy 15 from a total of 158 (9%) attending a specialist diabetes foot clinic may have had osteomyelitis. In 2008 again 158 patients were seen 12 had an MRI scan (7.5%) and 26 admissions occurred. This is not a true representation as some will have been diagnosed from the clinical presentation of bone in the base of an ulcer and / or plain X ray. Nor does this figure include those for whom a MRI was ordered when they were an in patient. Other reasons for attending the clinic also exist and for some of these there will be reasons for requesting MRI scans. It is known this will be primarily Charcot neuroarthropathy and also at least two patients had fractures and one a soft tissue syndrome. These three diagnoses were made only after the MRI scan. Using admission as a proxy 34 individuals were admitted to an acute General Hospital the prime reason would have been to administer intra venous antibiotics for 'severe' infection. These 34 individuals accounted for some 45 episodes of care with a range of number of admissions per individual being between 1 and 4. In 2009, up to the end of November the number of individuals seen was 177 with the number of visits ranging from 1 to 15. The total number of admissions was 45. The number sent for MRI scans was 22. None of the years has recognised those individuals who are

admitted direct from primary care, it only accounts for those who were admitted via the specialist Diabetes Foot Clinic.

Using Hospital Episode Statistics of amputations can also be used to act as a proxy for numbers with osteomyelitis and this too is not without its problems. Hospital Episode Statistics themselves are not without criticism. They have in the authors experience missed admissions related to diabetic foot disease when using the codes to trawl for patients to include in an audit. This under estimation, in this case of amputations, has also been recognized following a prospective study in a relatively stable population by Rayman *et al* (2004). Comparing their prospective data with data Hospital Episode Statistics collected using the International Classification of Diseases 9<sup>th</sup> revision between 4.2 and 8.7% of amputations were missed. This means we may be missing (and under treating) a large proportion of patients.

Some of the patients with osteomyelitis require hospitalization for diagnostic studies, surgery to remove infected or necrotic bone and / or lengthy (and usually parental) antibiotic therapy (Lavery *et al* 2009). Despite recent advances many patients with osteomyelitis fail with aggressive medical therapy and resort to surgical therapy (Haas and McAndrew 1996). An early diagnosis of osteomyelitis is essential to optimise therapeutic intervention (Tan and File 1999, Delcourt *et al* 2004, Zgonis and Roukis 2005). A precise diagnosis of osteomyelitis and the causative organism is clearly preferable when a prolonged course of therapy, usually antibiotics, or local debridement or amputation is advised (Grayson *et al* 1995, Crolle *et al* 1996).

The optimal approach to diagnosing (and managing) osteomyelitis in the foot in diabetes is unclear (Jeffcoate and Lipsky 2004). Delays in diagnosis occur because infection can mimic other medical conditions (see Charcot earlier) and often the classical clinical signs are absent particularly when associated with diabetes (Cunha 2002, Paluska 2004, Jeffcoate and Lipsky 2004). Lipsky *et al* (2004) suggest more than 50% of patients do not exhibit the classic local signs of pain and swelling or systemic signs of chills, fever, and metabolic disturbance (Paluska 2004). Tan and

File (1999) advocate searching for deep infection, notably osteomyelitis, when any suspicion of infection occurs in the diabetic foot. Typically in diabetes this would be suggested by deep plantar ulceration (Cunha 2002). Even in specialized foot care centres peripheral arterial occlusive disease and osteomyelitis still represent the greatest challenge in the strife for limb salvage (Kraus *et al* 2002). Jeffcoate and Game (2006) candidly say ‘the presence of osteomyelitis has major implications for limb salvage’, Cavanagh *et al* (2005), also bluntly, point out that sometimes ‘infection puts an individual s life at risk’. In this reported research the foot ulcer is taken to mean the common and classical ‘mal perforans’ ulceration.

If the infection extends into fascial planes and foot compartments it may ultimately result in serious systemic illness, excessive soft tissue loss or compromise the mechanics of the foot, any of which may make amputation the only option (Hill, Holtzman and Buse 1999).

## 2.4 Diagnosing osteomyelitis

The diagnosis of osteomyelitis underlying a diabetic foot ulcer is challenging as few clinical features are useful in making the diagnosis. Accurate and timely diagnosis of osteomyelitis is extremely important to prevent proximal migration of the infection and amputation (Kaleta, Fleischli and Reilly 2001).

### 2.4.1 Gold standard diagnosis

The definitive diagnosis of osteomyelitis is made using a biopsy to culture organism(s) from the site of infection (Wu *et al* 2007). The rate of positive diagnosis even with image guidance for biopsy sighting is reportedly low (41 of 75) (*ibid*). The fear of introducing infection and the need for a surgical practitioner to perform the



biopsy make the consideration of non invasive imaging strategies far more attractive (Kapoor *et al* 2007).

Bone specimens, obtained either percutaneously or at the time of surgery, may become contaminated, resulting in a false-positive test result, or the infected area may be missed during sampling (i.e. sampling error), resulting in a false-negative result (Dinh, Abad and Safdar 2008). Culture results may be false negative if the patient has recently had antibiotic therapy. Histopathological examination results may be false negative if the bone has evidence of necrosis or inflammation for other reasons (*ibid*).

The definition of what constitutes a reliable microbiological test in osteomyelitis remains controversial (Hartemann-Heurtier and Senneville 2008). It has been suggested that the results of superficial sample cultures do not correlate with those of bone (Wheat *et al* 1986 and Sutton *et al* 2000). Wheat *et al* (1986) performed a prospective study on one hundred and thirty one infections in patients with diabetes and foot wounds. The enquiry examined individuals admitted to an acute teaching hospital and the final analysis was on fifty four infectious episodes. This reduction in numbers was due to specimens that were unable to be taken without contact with the ulcer or other openly draining lesions and as such were classified as unreliable. A comparison of organisms found in reliable and unreliable specimens showed that reliable specimens were more closely related to clinical findings. The work by Sutton *et al* (2000) was a retrospective review of diabetes associated suspected bone infection. The definition of a positive result for osteomyelitis was in-precise in that it was by either positive bone culture or histology or imaging and prolonged follow up. This poor association has also been confirmed in a study by Senneville *et al* (2006) where surface swab culture and bone fragments taken transcutaneously via normal skin had a low correlation of results. This study was on individuals in whom no antibiotic therapy had been used for the four weeks preceding biopsy which although ideal it is rarely achievable. This is because of the ethical dilemma of not treating a

clinically evident infection. The superficial ulcer cultures were included if taken at least three days prior to biopsy. This low relationship maybe in part due to the surface swab finding skin commensal bacteria, the deeper tissue sampling showing the obligative and facultative anaerobes found in deep tissue infection. No studies appear to have compared the two methods available for bone sampling – transcutaneous needle aspiration and percutaneous bone biopsy.

#### 2.4.2 Other tests used clinically

As a result of the paucity of accuracy in diagnosis has often been made on a combination of good clinical judgement and tailored investigations (Lipsky 1997). To make it more confused Delcourt *et al* (2004) maintain that diagnosis is often difficult clinically but Paluska (2004) agrees with Berendt and Lipsky (2004) suggesting the diagnosis is primarily a clinical one. Treece *et al* (2004) agree with the basis being clinical stating ‘because there is a relative scarcity of scientific evidence on which to base decisions management programmes are to a large extent defined by the experience and skill of the individual clinician, as well as their preconceptions, available resources and the conventions and constraints of local health care’. Although it should be remembered clinical judgment may be misleading in the low grade infections due to lack of clinical manifestations as noted and discussed by Newman *et al* (1991) and Delcourt *et al* (2004).

The controversies of not having a precise definition and with that a firm diagnostic criteria have been discussed in section 2.2

The most definitive work so far has been by The International Working Group on the Diabetic Foot. They have published a paper headed by Berendt *et al* (2008) on ‘Diabetic foot osteomyelitis: a progress report on diagnosis and a systematic review of treatment’ in which a diagnostic algorithm is proposed. This is in Appendix 10.8. This algorithm has yet to be validated. It includes criteria of bone biopsy, MRI, plain

X ray, Erythrocyte Sedimentation Rate (as the only inflammatory blood marker) and clinical signs and symptoms.

#### 2.4.3 Imaging techniques in the diagnosis of osteomyelitis

It has been suggested that the use of imaging studies may help diagnose or better define and detect pathological findings in bone (Lipsky *et al* 2004). However the many diagnostic tests offered often yield equivocal results (*ibid*). Great care must be exercised in determining the usefulness of the imaging tests as, of necessity; they have been evaluated in patients selected on the grounds of clinical suspicion. This means the results are heavily influenced by the pre-test probability of the presence of disease (Wrobel and Connelly 1998). Although the need for well designed studies with a post-test diagnostic probability has been recognised these do not appear to have been performed (Lipsky *et al* 2004). Additionally many of the studies that are set up to assess the diagnostic characteristics of these imaging tests have limitations regarding the choice of reference test to conclusively establish the diagnosis of osteomyelitis (Dinh, Abad and Safdar 2008).

Plain radiographs have the advantage of being convenient, inexpensive and reproducible (Paluska 2004). However early in the disease process plain radiographs will be normal, (Longjohn, Zionts and Scott 1995) or, not clearly differentiating between both infection and Charcot joint (Grayson *et al* 1995). The osteolytic changes of bone loss are not evident on plain film until between 30% and 50% of bone mineral has been removed (Waldvogel, Medoff and Swartz 1970, Paluska 2004). Jeffcoate and Lipsky (2004) reckon on this being at approximately 4 weeks post disease. The differences in reported sensitivity, 43%-75%, and specificity, 75%-83%, suggest that this should not be used as a definitive diagnostic test (Boutin *et al* 1998 and Tumeh and Tohmeh 1991 both cited in Paluska 2004). False positive plain film X rays have been noticed to be associated with neuropathic joints, degenerative or inflammatory arthritis (Crim and Seeger 1994).

Magnetic Resonance Imaging (MRI) is used commonly as the gold standard in the United Kingdom (where bone biopsy although the accepted criterion is rarely used, Jeffcoate and Lipsky 2004) to recognise infection because it presents both a detailed accurate image. This includes areas of soft tissue oedema and bone abscesses (Mader, Cripps and Calhoun 1999). MRI reveals active medullary osteomyelitis as an area of abnormal marrow with altered signal. Marrow oedema gives the same altered signal and as such reduces the specificity because of other disease processes such as acute Charcot neuroarthropathy.

Sartoris (1994) has performed a meta analysis on ten previous studies to assist recognition of abnormal soft tissue signals with MRI. It is also more sensitive and precedes these changes than plain film or simple Computerised Tomography (Crim and Seeger 1994). MRI can be used to give good structural visualization and spatial resolution (Paluska 2004), superior contrast resolution and a multiplanar examination (Flemming, Murphey and McCarthy 2005). Reported sensitivities are high ranging from 88%-100% with a specificity of 53%- 94% (*ibid*). It is recognised that specialist technical and interpretive skills are required to achieve these levels (Berendt and Lipsky 2004).

This puts forward the suggestion of another test, for example inflammatory blood markers, being used in addition. Another meta-analysis of MRI studies has shown that the procedure performs well in the diagnosis of osteomyelitis of the foot and ankle in adults (Kapoor *et al* 2007). This study was well conducted in that it acknowledges the problems associated with the combination of the studies; notably using English language only articles, few studies followed the assessment of individuals with scan results read blind to other diagnostic techniques or with biopsy as a confirmatory tool, however the presence or absence of Charcot foot was not typically documented and it is recognised that this is one of the potential differential diagnoses particularly in the diabetic population. The advancements made in the technique are also acknowledged as possible ways of diagnosis being made with greater certainty in the more recent studies, for example the use of Gadolinium as a

tracer in suspected infection and the use of secondary diagnostic signs such as cortical breaks in diagnosis of Charcot neuroarthropathy.

Nuclear medicine techniques alone usually are considered to be either bone scans or leukocyte scans. Nuclear medicine techniques of leucocyte scanning involve the introduction of a nuclear label by intra venous injection. The label, or tracer, has been developed so that it attaches to anti-granulocyte monoclonal antibodies in the blood. The diagnosis is made by the recognition of areas of inflammation, recognised by increased blood flow, and hence uptake of the marker, has been found not to be particularly sensitive. Bone marrow cannot be visualized directly causing difficulty in determining the extent of disease (Sartoris 1994). Boorgula *et al* (2004) looking at 34 patients reckoned on a 55.55% sensitivity with a 11.7% false negative result which is not acceptable in a disease process that can have such rapid deterioration and devastating consequences. The false positives can be due to neuropathic joint changes and cellulitis – both very common in the diabetic foot (Crim and Seegar 1994). It has been proposed the false negative result level can be overcome and ‘confirmation of a negative result with confidence’ be given if a fourth scan is taken at 24 hours after the initial introduction of the marker (Jones, Chalmers and Dunlop 2001, Rubello *et al* 2004.) Rubello *et al* (2004) also suggest however that ‘if evidence of a high uptake intensity in the early LeukoScan imaging was a strong indicator of infection and delayed imaging in these cases did not improve specificity’. This study to provide this evidence against the fourth scan was performed on some 220 patients 78 of who had diabetes and an associated infected foot ulcer. The sensitivity did not change from 91.9% but increases were noted in the specificity from 75.0% to 87.5%, the negative predictive value from 70.5% to 73.6% and the positive predictive value from 93.4% to 96.6%.

All bone scans are recognised to give false negatives in patients with markedly diminished blood flow to the extremities (Eckman *et al* 1995, Embil 2000) which is known to be the case frequently in diabetes. Early changes are picked up but are not specific to infection (Kapoor *et al* 2007). False positives also occur because of the similar uptake in both Charcot neuroarthropathy and infection (*ibid*). White blood

cell scans are not easily interpreted to differentiate soft tissue infection from bone infection (*ibid*).

Computerised Tomography (CT) is a well established technique that allows examination of bones and joints in any anatomic plane required (Flemming, Murphey and McCarthy 2005). It is more sensitive to cortical bone changes than plain X Rays (Crim and Seeger 1994). Used alone its sensitivity is reduced from 87% in the calcaneus to 25-33% in the mid foot where many diabetic foot lesions occur (*ibid*). When the transition from infected to healthy bone extends over multiple layers of scan it has been argued from a clinical view point that a degree of subjectivity is required to predict the proximal margin of disease (Sartoris 1994).

Bone scintigraphy and labelled white cell scans are sensitive in the detection of bony pathology but the nature of complex three dimensional anatomy of the foot means localization of the abnormality has in the past been difficult (Fielding *et al* 2006). The new hybrid Single Photon Emission Computed Tomography / Computer Tomography (SPECT/CT) offers the opportunity to combine the functional information of nuclear medicine with the accurate anatomical information provided by CT (*ibid*). The multi planar images can then be reconstructed mathematically to produce a three dimensional set of images (Gemmell and Staff 1998). The diagnostic information is provided by the action of the injected pharmaceutical drug; a radioactive marker. The radioactivity is simply to allow recognition of the localization of the drug and as such is provided by a very low dose (Sharp 1998).

#### 2.4.4 Reference test for diagnosis

To confirm the presence of osteomyelitis in the proposed research an imaging technique will be used. The imaging techniques for diagnostic approaches suggested by Berendt and Lipsky (2004) are plain radiograph alone in many cases despite its poor sensitivity and Magnetic Resonance Imaging (MRI) (in preference to isotope scanning due to the better sensitivity and specificity). Paluska (2004) suggests the

use of plain radiographs, nuclear medicine testing, Computerised Tomography (CT) or MRI. Despite apparent confusion and contradiction over the choice of technique due to the highest sensitivity and specificity being obtained by using a MRI scan this will be used to as the reference test to recognise infection in this study reported here. Diagnostic sensitivity has been reported to be between 90% and 100% by (Eckman *et al* 1995, Crolle *et al* 1996, Craig *et al* 1997 and Enderle *et al* 1999). The problem is distinguishing between other causes of bone marrow oedema. Nevertheless Enderle *et al* (1999) report positive and negative predictive values of 93% and 100% respectively.

## 2.5 The immune response

The immune response developed in reaction to the infection of osteomyelitis, like all infections, is as for that to foreign substances – it is acquired immunity. Acquired immunity involves two parts; the humoral immunity involving antibody production plus complement and cell-mediated immunity. Complement is a serum enzyme cascade usually activated by antibody-antigen complexes; the enzymes released are capable of lysing cells and bacterial cells to which antibody is attached.

The humoral response includes the production of immunoglobulins. Immunoglobulins are glycoproteins present in serum and tissue fluid produced on exposure to antigen.

Neutrophils are the characteristic cells of acute inflammation playing an important role in non-specific immunity engulfing and digesting micro organisms.

Complement is a series of plasma proteins that become bound to antibody-antigen complexes in a specific sequence (cascade) which contributes to humoral immunity in two main ways. Firstly the binding of complement and/or antibody to the surface of a micro organism attracts phagocytes. This binding is sometimes called

opsonisation, opsonisation facilitates phagocytosis. Phagocytes are cells that engulf and digest foreign material e.g. bacteria. Secondly complement fixation, the fixation of complement to the surface of a micro organism results in opsonisation and if the complement cascade is completed lysis of the cell. (This binding can occur by two major routes – the classical and alternative pathways.)

Cell mediated immunity involves the activation of T cells where antibody plays a subordinate role. The activation of lymphokine mediators from T cells includes the production of chemotactic leucocyte factors; this process of cell attraction includes the recruitment of Neutrophils into the area. Neutrophils are one division of white blood cells (Leucocytes) of which they constitute approximately 60%. They are sometimes termed polymorphonuclear granulocytes. They bear surface receptors for Immunoglobulins A, G and complement components. The importance of this reaction in the situation of osteomyelitis is the interaction of the factors with cells is the elimination of bacteria resistant to phagocytosis.

## 2.6 The acute phase reaction

This is a term that is used to describe a complex range of physiological changes that happen following inflammatory tissue damage including trauma, infection and burns. There are haemodynamic changes including a leucocytosis and changes in the concentration of many plasma proteins plus systemic effects seen when an individual becomes pyrexia. This includes a rise in complement proteins and immunoglobulins. Note that the reaction and production of these changes are not specific to infection.

Quantitative measurements of acute phase proteins can be useful in noting the presence and extent of inflammation. As the markers are markers of inflammation and are non - specific to infection any individual with a disease known to be associated with inflammation was excluded in this research.



The measurement of this reaction can be performed by the examination of the blood for changes in cellular concentrations e.g. Leucocyte count, and specifically in this study Neutrophil Count, the changes in plasma proteins affecting the Plasma Viscosity e.g., fibrinogen, the release of chemicals that play a part in the inflammatory process such as C Reactive Protein.

The speed of reaction of the different components of this process is of variable onset. The time taken for the different reactions to peak and decline makes the measurement process involved. The complexity of the recognition of the different stages of the inflammatory, and hence healing and repairing processes, can be determined using different markers of the process. As the markers are not specific to the different types of injury they are often termed 'non specific'.

## 2.7 Inflammatory blood markers; non specific

Biomarkers of sepsis are clinically useful only if they fulfil certain requirements. These are clearly related to the therapeutic consequences of either treating or withholding treatment. The requirements are in part related to the biochemical properties of stability, half-life of induction and elimination, range of concentration and relationship to disease severity, response to therapy and prognosis (Meisner 2005).

Questions have been raised about the response of people with diabetes to develop these inflammatory markers (Armstrong *et al* 1996, Leichter *et al* 1998, Oncul *et al* 2006). It has been recognised that the cardinal signs of infection can be both mimicked and obscured by the diabetes associated complications of ischaemia or neuropathy (Cavanagh *et al* 2005). The changes in the microvessels affect both delivery of the cells required and the inflammatory response (Sannomyia, Pereira and Garcia-Leme 1990). It is well recognised that diabetes as a disease process affects both the circulatory and nervous systems (Edmonds *et al* 1986). The effect

this may have on the ability to produce inflammatory markers has not been fully examined.

The work that has previously been published on the potential confounding factors within diabetic foot disease has not been detailed in its definitions of the confounding disease processes. The lack of clarity as to the way in which the diagnosis of confounding disease was made means that this current work was not able to be fully compared against previous work. This lack of clarity is found for disease processes such as cardiovascular and peripheral vascular disease and neuropathy. All are reported to be causative in potential effects on inflammatory blood markers yet there is no information on how the disease was defined or diagnosed in many of the studies.

Each marker that had potential to be used within this study is now discussed. The previous studies found using non-specific inflammatory blood markers in diagnostic testing have been used mainly to monitor either systemically unwell patients with sepsis and multi organ failure or paediatric patients in the recovery process from septic arthritis and bone infection. Those looking at the disease process in adults have focused on the vertebral bones (Kemp *et al* 1973, Caragee, Kim and van der Vlugt 1997, Cunha 2002) where Mader, Shirtliff and Calhoun (1997) suggest it is the most common site. All suggest this form of osteomyelitis is chronic or sub-acute, similar to that occurring in the foot in diabetes. Kemp *et al* (1973), Frederickson, Yuan and Olans (1978) and Caragee, Kim and van der Vlugt (1997) all suggest the use of multiple markers.

Four papers only Upchurch, Keagy and Johnson (1997), Kaleta, Fleischli and Reilly (2001), Cunha (2002) and Jeandrot *et al* (2008) have specifically looked at typical, or classic, diabetic foot ulceration and associated osteomyelitis despite its frequency and potential to be associated with increased morbidity, not least of which is lower limb amputation, and morbidity, as well.

### 2.7.1 Neutrophil count

As opposed to a broad count of all Leucocytes the sub group of Neutrophils was examined for potential. The reasoning behind looking at Neutrophils in particular is that their prime function is to ingest and kill bacteria. As the cause of osteomyelitis is most commonly bacterial infection this is clearly rational. There does not appear to be any influence on the ability to develop an increase in white cells when infection requires the mobilisation of these types of cell that may influence the use of this test with the exception of known disease of the white cells themselves.

The use of raised Leucocyte count is regarded as sensitive but not specific indicators for the presence of infection in paediatric patients (Jackson and Nelson 1982, Hiew, Tan and Cheng 1992 and Unlika-Kallio *et al* 1994).

Li *et al* (2004) have used raised Leucocytes to examine septic arthritis in adults retrospectively. They found a poor sensitivity missing approximately 50% when looking at Leucocyte count alone. The confirmation of an infected joint in the Li *et al* (*ibid*) paper was by positive culture from joint fluid aspirated or frank pus in arthroscopy.

It should be noted that defects in leucocyte chemotactic factors have been observed when a poor inflammatory response occurs (Bagdale, Root and Bulger 1974, Tan *et al* 1975 and Molenaar *et al* 1976). The complications associated with chronic hyperglycaemia that are the prime problems of diabetic foot disease, namely neuropathy and ischaemia, are compounded by the metabolic derangement known to be aggravating chemotaxis in the polymorphonuclear leukocytes (Bagdale, Root and Bulger 1974). In their review Molenaar *et al* (1976) also recognise decreased cell adhesiveness and cell movement, decreased bacteriocidal activity and possible deficiencies in serum complement in individuals with diabetes. Certainly from clinical experience it is apparent that the classic response associated with infection is noted to be reduced to that expected for a comparable degree of tissue damage.

Armstrong *et al* (1996a) suggest 54% of patients with a diabetic foot infection diagnosed clinically have a normal white cell count, with Leichter *et al* (1998) reporting only 25.5% having a raised count in infection. This reduced white cell count maybe due to their compromised systemic response to infection (Caragee *et al* 1997, Lewis *et al* 1998, Jeffcoate and Game 2006). Armstrong *et al* (1996a) and Armstrong *et al* (1996b) describe these finding in patients that have been admitted to hospital as a result of the infection which may suggest it is 'severe'. In the later paper it is also recognised that there was no stratification by type of infection which they suggest may be soft tissue alone versus osteomyelitis. A definition of 'severe' is missing. Lipsky *et al* (2007) describe a prospective study in which an elevated white cell count was found to be associated with failure to treat adequately an infection after ten days in-patient treatment with antibiotics. This study appears to be robust in that it was a prospective design with a large number of patients at 402 but regrettably it excluded those with osteomyelitis so is of limited applicability to this proposed study. Critical limb ischaemia was also not included and this is certainly of concern to those involved in the care of the diabetic foot as it is strongly associated with limb loss.

Only two studies to date have been found which has looked at Neutrophil levels (Pittet *et al* 1999 and Al-Gwaiz and Babay 2007). This first study was a retrospective cohort study of diabetic foot infections. Retrospective studies can be considered to have a selection bias result by the inclusion of the sicker patients with an abnormal baseline parameter. The Neutrophil Count in this series was not among the independent factors that predicted treatment failure. The study group was patients admitted because of their foot problem and as such do fit the suggestion of more poorly patients in retrospective work. The cell count however was not looked at in isolation but as an indicator of 'sign of infection' together with the temperature of individuals. The total number of individuals for whom this complete information (temperature and Neutrophil Count) was available from a total study population of ninety-one was only twenty-nine.

Al-Gwaiz and Babay (2007) appear to have studied patients who were hospital in-patients with a confirmed bacterial infection (by culture). It is unclear if this is blood cultures or of tissue e.g. bone culture. The assumption that the patients were in patients follows from the statement about them having bacterial cultures performed, this would be most unusual in an out patient setting. This study was carried out to determine the diagnostic usefulness of three different methods of examining the effect of bacterial infections on Neutrophils which are in the statement below. They state that 'the relationship between acute infectious diseases and white blood cell count (WBC), Absolute Neutrophil Count (ANC) and an increased number of band neutrophils has been recognized for many years' but do not substantiate this with any references. They provide an analysis suggesting sensitivity of the different methods including a column for 'severe' infections but this term is not defined in the text. The lack of detail in this paper does not assist in the usefulness of using it as a comparative piece of work.

The method of analysis used for monitoring Neutrophil activity in this thesis study was absolute count. This is in line with the paper by Al-Gwaiz and Babay (2007) who following the analysis of different evaluations of white blood cells found absolute count the most sensitive in predicting bacterial infection. This was in the population of age 1 year to 70 years and compared absolute count, band cells, Döhle bodies and neutrophil vacuoles.

The fact of osteomyelitis being an infectious disease suggested the use of Neutrophil count would be prudent so it was included in the final choice of markers.

### 2.7.2 Haemoglobin

Diabetes as a chronic disease is likely to produce an acquired anaemia non- immune anaemia (Al-Khoury *et al* 2006), in addition to the effect of renal disease affecting the production of erythropoietin and as such produce anaemia. Anaemia associated with chronic disease is a normochromic normocytic variety. The presence of chronic kidney disease has been shown to potentially increasing the prevalence of anaemia by a factor of four (*ibid*). Diabetes alone, i.e. without recognised kidney disease, may

account for some 16% of patients with anaemia (O'Mara 2008). The O'Mara paper used that definition of the National Kidney Foundation. In chronic renal disease a reduction in erythropoietin production leads to a reduction in erythrocyte production, and a normochromic normocytic anaemia. The anaemia caused by infection is that of chronic disease.

Anaemia has been recognized previously in patients with diabetes and found to be more complex and multi-faceted using a hierarchical study review of ten papers (Balshaw-Greer, Davies and Casey 2005). This is particularly so for those with the complications of albuminuria and reduced renal function (Thomas and Rampersad 2004).

Leichter *et al* (1988) in a series of 55 patients with diabetes and serious foot infections, clinical anaemia measured by Haemoglobin level was not found to be present. There is no definition of what they consider to be 'serious' or 'anaemia'. The impact of renal function is not mentioned in detail with the only sentence used stating 'tests of renal function reflected mild to moderate abnormalities for the group'.

Furthermore a long term reduction in Haemoglobin level, but not to a level diagnostic of anaemia, has been found in people with diabetes (Craig *et al* 2005). Thomas *et al* (2003) in a study of 820 patients found an undiagnosed prevalence of 23% using the WHO definition. This was comparing these people with diabetes to the general population and comparable renal disease and iron stores. As no level of Haemoglobin is definitive for people with diabetes to be either normal or abnormal the measurement of its level will continue to be used in the proposed research. The mean age of this Thomas study group was likely to be similar to that in the proposed study at 62.2 years and with 95% of Caucasian decent.

The recognition of the possible effect suggested by Ting *et al* (2006) of Metformin causing a reduction in serum Vitamin B<sub>12</sub> and as such anaemia its use was noted.

(The Adetunji *et al* (2009) paper discounting this effect came out after data collection was complete.)

The definition of anaemia in studies can be seen to be variable. However a level of less than or equal to 11g/dl (irrespective of gender) has been suggested in guidelines to show benefit to patients if improved (National Kidney Foundation 2001). The gender specific definition of anaemia by the World Health Organisation (WHO) (1968) is less than 13g/dl in men and less than 12g/dl in women.

The measurement of Haemoglobin as an indicator of an infectious chronic disease – osteomyelitis led to its inclusion in the study. Renal anaemia was attempted to be excluded by excluding individuals on known renal replacement therapy or with two eGFR results less than 60 ml/min within the preceding 6 months to consent.

### 2.7.3 C Reactive Protein

C Reactive Protein is an immunoglobulin which in an antigen complex triggers the complement cascade. It is called C Reactive Protein as it has the property of being able to bind to a polysaccharide in the cell wall of pneumococci bacteria, the fraction of the wall being fraction ‘C’. The bacterial polysaccharides and phospholipids released by the damaged tissue also become activators of the complement pathway. At the cellular level C Reactive Protein initiates opsinisation and phagocytosis of invading cells. It also binds to and detoxifies endogenous toxic substances produced as a result of tissue damage. Levels are not influenced by anaemia or heart failure. It is highly sensitive due to large incremental changes.

C Reactive Protein is known to be higher in the population of people with diabetes as a background level (Sattar 2006a). This work has not however differentiated what, if any, effect foot ulceration (and this with / without infection) has on the level. The work is recognition of diabetes as a cardiovascular disease.

C Reactive Protein is triggered to rise 'several hundred fold' (Kallio *et al* 1997) by tissue damage that maybe either infectious or ischaemic (Khachatourians *et al* 2002).

Unlika-Kallio *et al* (1994) claim C Reactive Protein is a better marker than Erythrocyte Sedimentation Rate (in paediatric patients) in bone infection. C Reactive Protein level is a rapid indicator of inflammation and tissue necrosis (Unlika-Kallio, Kallio and Petola 1994). The rapid development of C Reactive Protein is a reason for its preferred use making it a 'better' marker so say Petola and Räsänen (1982). They used C Reactive Protein to assist in the diagnosis of osteomyelitis in children. Roine *et al* (1997) state C Reactive Protein is a better predictor of sequela prone paediatric patients.

Upchurch, Keagy and Johnson (1997) have demonstrated a rise in C Reactive Protein in people with diabetes and associated foot ulcers when cutaneous infection is present. What is not clear in this small study is whether any investigation, or indeed suspicion, of the presence of osteomyelitis was considered. Where there is the potential for medication to have an affect on the result no detail about either dosage or length of use has been reported. Medication that may cause an alteration in the result was simply noted as being prescribed. Metformin is the most likely cause where it has been suggested it may have a specific interaction involved in the synthesis or secretion of C Reactive Protein (Carter *et al* 2005). It is likely that patients within the proposed research are being treated with this drug. In a double-blind placebo controlled trial Carter *et al (ibid)* found statistically significantly lower levels of C Reactive Protein after 12 and 24 weeks treatment with Metformin after starting with similar levels of marker and similar levels of metabolic control of the diabetes as a disease. This was in 'overweight' individuals. Body Mass Index is not routinely considered in the diabetes foot clinic and was not available for all the individuals as many had their diabetes care in primary care alone – the diabetes database is for specialist care only. This study used the same definition as Carter *et al (ibid)* in defining overweight as a BMI greater than 25 kg /m<sup>2</sup>.



In this Carter *et al* (*ibid*) study the fact that acute infection can raise the level was recognised and individuals with a level of  $> 10$  mg/l were excluded from the analysis. This alteration in C Reactive Protein has been noted before in a trial by Chu *et al* (2002) that was set up to look at the reduction of cardiovascular risk factors that C Reactive Protein is considered to be one of. No detail of the medication dose was given in this paper by Carter *et al* (2005) or that by Chu *et al* (2002). This means it is difficult to consider this as a potential confounder to results in papers that follow.

Diabetes as a disease is itself recognised to be a risk factor for cardiovascular disease. Cardiovascular disease and diabetes together have been further studied with C Reactive Protein. The increase in C Reactive Protein is also noted with Insulin resistance (Nesto 2004) – a metabolic syndrome associated with type 2 diabetes in particular. Chronic subclinical inflammation is a component of the Insulin resistance, or metabolic, syndrome in which cardiovascular risk factors including type 2 diabetes occurs (*ibid*). He does however make the point that the raised level is actually the upper end of normal and may not be detected by the usual assay methodology.

Considering peripheral vascular disease as a variant of cardiovascular disease and recognising that it too is common in diabetes a study by Yu *et al* (2004) has shown by multivariate regression analysis that higher serum levels of C Reactive Protein and longer duration of diabetes (with no definition to support this expression) were both independently associated with peripheral vascular disease in type 2 diabetes. The study was cross sectional and involved 30 patients with vascular disease as defined by the use of an Ankle: Brachial Pressure Index (ABPI) and comparing them to 60 with what was considered to be normal. The level of inflammatory marker was statistically significantly raised but the methodology can be questioned. The use of an Ankle: Brachial Pressure Index is not always satisfactory in people with diabetes due to vessel calcification producing false high readings suggestive of normality when in fact disease is present.

Recent predictive models that appropriately account for the possible confounding factors within coronary heart disease and C Reactive Protein levels ‘the magnitude of the association is considerably attenuated towards the null’ (Lawlor *et al* 2005). The authors suggest that the marker should be used to predict coronary heart disease but may not be casually related to it. So it maybe, that this confounding factor can be discounted when looking at individuals with known cardiovascular disease. However more recently it has been suggested that ‘evidence that C Reactive Protein does not cause cardiovascular disease does not mean that inflammation plays no role in atherosclerosis’ – part of lower extremity peripheral vascular disease (Davey-Smith, Thompson and Lawlor 2006). These findings support, albeit not strongly, the abnormal levels of C Reactive Protein found generally in people with diabetes with associated complications discussed above.

Jeandrot *et al* (2008) again studied in patients as opposed to out patients with the study attempting to differentiate foot ulcers with bacterial colonisation from infection. The study, on antibiotic naive patients, is difficult to justify ethically when infection is suspected. Osteomyelitis is not always obvious clinically so the results of this study maybe of limited comparability to the currently reported study. It is not clear how those with diabetes were used as controls were either included or excluded with the other known factors that can raise inflammatory markers. The assay method for C Reactive Protein was different to that available routinely as it was able to measure levels below 5mg/l.

Generally C Reactive Protein is thought to be more specific for infection than Erythrocyte Sedimentation Rate (Haas and McAndrew 1996, Wall 1998).

Lew and Waldvogel (1999) have recognised the need to distinguish people with diabetes as a sub group clinically. They state the need to consider this set because of the important contributing factors of the metabolic consequences of the disease process, poor blood supply, bone ischaemia and neuropathy all adding to bone destruction.

C Reactive Protein is routinely used in the Royal United Hospital, Bath for monitoring response to antibiotic therapy so it was included to determine if it was of use in recognising osteomyelitis. The potential confounders including cardio and peripheral vascular disease were noted as was the use of Metformin.

#### 2.7.4 Erythrocyte Sedimentation Rate

There are multiple influences on Erythrocyte Sedimentation Rate include age, fluid balance, nutritional status, and hormonal changes (Khachatourians *et al* 2003) plus others that alter the fibrinogen levels including diabetes and significantly, as they are associated with diabetes, heart disease and end-stage renal failure, (Kaleta, Fleischli and Reilly 2001). It is uncertain if anti-inflammatory medication decreases Erythrocyte Sedimentation Rate (Bridgen 1999). Bridgen (1999) cites Sox and Laing (1986) when he includes obesity in his list of influences but says it is 'not of clinical significance'. This is because Erythrocyte Sedimentation Rate reflects the concentration of fibrinogen and immunoglobulins in the plasma (Kallio *et al* 1997). Obesity is in part causative of type 2 diabetes, the most common form of diabetes and as such this may be of note. The study however was not set up to consider Body Mass Index as part of the protocol. It was however collected post study to look at any potential correlation with C Reactive Protein but many individuals did not have the data available as their diabetes care was in primary care and the information not available to the researcher.

The use of raised Erythrocyte Sedimentation Rate is regarded as sensitive but not specific indicators for the presence of infection in paediatric patients (Jackson and Nelson 1982, Hiew, Tan and Cheng 1992 and Unlika-Kallio *et al* 1994).

Li *et al* (2004) have used raised Erythrocyte Sedimentation Rate to look at septic arthritis in adults retrospectively. They found a poor sensitivity 10% having a 'relatively low or normal' level.

Kaletka, Fleischli and Reilly (2001), Berendt and Lipsky (2004) and Berendt *et al* (2008) advocate the use of an indicative raised level of Erythrocyte Sedimentation Rate at 70mm/h. Berendt and Lipsky (2004) declare that above this level is specific for infection. But only ‘possibly’ diagnostic for osteomyelitis being when there is ‘no other plausible explanation’ (Berendt *et al* 2008). Possibly being taken to mean ‘but on balance less rather than more likely’.

In his study of elderly patients with diabetes Cunha (2002) suggests with no rationale that an Erythrocyte Sedimentation Rate of greater than 100mm / hour is an inexpensive but non – specific test of osteomyelitis.

Erythrocyte Sedimentation Rate was not included in the final study as it is not a standardised quality controlled test.

#### 2.7.5 Plasma Viscosity

The use of Plasma Viscosity as opposed to Erythrocyte Sedimentation Rate is more practical in that using a standardised laboratory test ensures quality control. It also has the advantages of not being affected by anaemia (one of the potential confounding factors) and is abnormal earlier than Erythrocyte Sedimentation Rate (Fleck and Meyers 1995). Additionally there is little effect on the level due to age. The only age limitation in the presented work was to be over 18 years old. Plasma Viscosity is affected by the proteins of large molecular size including fibrinogen and some immunoglobulins. The results are only slightly changed with age (as fibrinogen increases), with no differences between the genders and as previously stated independence from the effects of anaemia, plus they are available quickly.

Plasma Viscosity has been seen to rise rapidly after the onset of infection (Paluska 2004). This paper was about osteomyelitis but not specifically as a complication of diabetes associated foot lesions. However it does have a section dedicated to the problems of osteomyelitis in diabetes.

Plasma Viscosity has been shown in patients with type 2 diabetes to be 'abnormal', that is raised above what is considered 'normal' (MacRury *et al* 1990, Coppola *et al* 1997). The first study, MacRury *et al* 1990, compared controls (no known diabetes) to individuals with known diabetes. Some with diabetes had known neuropathy diagnosed in a 'normal' clinical fashion plus 'fancy' electromyography studies. The individuals with known diabetes were a mixture of type 1 and type 2, some of the type 2 individuals having Insulin therapy. It was found all people with diabetes had a higher, statistically different ( $p < 0.001$ ) Plasma Viscosity. Interestingly those with diabetes and a diagnosis of neuropathy did not have a greater mean Plasma Viscosity compared to those with diabetes and no neuropathy. The number of participants with diabetes was small ( $n=39$ ) and matching occurred within the diabetes known group for type of diabetes, age and gender. Other known causes of neuropathy were excluded and the controls were 'free from pain or paraesthetic symptoms in their legs with a normal fasting blood glucose'. The planning shows some well considered thoughts. Trauma in patients with neuropathy is the major cause of diabetic foot ulceration that leads to osteomyelitis and as such considering the presence of nerve damage is prudent.

The second study, Coppola *et al* 1997, was also based on small numbers ( $n=15$  healthy individuals i.e. not having a diagnosis of type 2 diabetes, and  $n=15$  of each diabetes and free from cardiovascular complications and with both clinical, and measured, evidence of coronary or peripheral vascular disease and  $n=15$  with diabetes and cardiovascular complications) and of a cross sectional design. The study was well designed in the fact that the individuals in the study were matched for age and Body Mass Index. The majority of patients that attend the Diabetes Foot Clinic have type 2 diabetes and a majority will have peripheral vascular disease as part of their diabetic foot complications.

The Coppola *et al* (1997) study used extensive methods to determine the presence of cardiovascular disease including history, clinical examination, Electrocardiogram, exercise stress testing and döppler ultrasonography. The study presented in this thesis used only patient history and medication (using drug records) as an

assumptive method of the presence or absence of cardiovascular disease. The Coppola *et al* (1997) study acknowledges the limitations of the study being cross sectional and having only a small number of participants. It is also recognised that the small numbers mean that any cause and effect relationship between type 2 diabetes and alterations within the blood profile cannot be identified in diabetes related vascular disease.

The study by Coppola *et al* (1997) investigating the effect of Insulin appears to be adding Insulin to blood samples and not considering Insulin as a medication. How applicable this is to the use of Insulin as a drug to control diabetes is unclear. The conclusion of the study was that ‘the beneficial effects of Insulin on blood viscosity are not evident in type 2 diabetic patients, especially those with vascular complications’.

Recognition of the potential confounding factors suggested in these two papers was provided for in the data collection sheet.

#### 2.7.6 Procalcitonin

Procalcitonin (PCT) is a 116 amino acid propeptide of calcitonin synthesized in the parafollicular C cells of the thyroid that has only recently been used as a potential marker for bacterial infection and associated sepsis. Procalcitonin levels had been noted to rise only during severe infections with systemic manifestations to over 100ng/ml (Reinhart, Karzai and Meisner 2000).

Procalcitonin is under normal metabolic conditions produced and secreted by the C cells of the thyroid. In severe bacterial infections and sepsis the origin is extrathyroidal. In marked inflammatory conditions the principal source is ‘nonneuroendocrine parenchymal cells e.g. lung, liver, kidney, fat, muscle and stomach’ (Müller *et al* 2008). The normal level is very low (< 0.5 ng/ml). In microbial infections the amount of Procalcitonin can be seen to increase ‘up to a hundred times’ (Hladik *et al* 2005). A meta analysis comparing C Reactive Protein

and Procalcitonin has shown Procalcitonin to be superior in the diagnosis of bacterial as opposed to viral or non bacterial infection (Simon *et al* 2004). This meta analysis was of work performed on studies looking for bacterial infections in hospitalized patients. It was well conducted with specific inclusion and exclusion criteria with clear admissions of the limitations. Despite it being based around in patients some of the information it gives may be of use in comparison to this current study. Other factors known to raise Procalcitonin levels which may also cause other markers to rise include neuro endocrine tumours, non infectious inflammation, general sepsis and trauma. (There is no elevation of the levels of the mature calcitonin in inflammatory induction.)

Sitter *et al* (2002) have shown Procalcitonin not to be effected by renal disease when comparing results for the determination of bacterial infection or auto immune disease. The individuals in this study were classified according to their stage of renal disease and also taken into consideration was any immuno suppressive medication taken and auto immune disease. This study showed a lack of effect of renal disease on Procalcitonin echoes the work of Eberhard *et al* (1997) who looked at the use of Procalcitonin in the comparison of active auto immune disease and invasive bacterial infection. As renal impairment is a complication of diabetes that is often seen at the same stage of the disease as diabetic foot syndrome this is potentially very useful. Procalcitonin as a marker is not affected by immunosuppression or neutropenia.

Stucker *et al* (2007) when examining the utility of Procalcitonin as a predictor of infection in elderly hospitalized patients (their definition of elderly being over seventy five the study mean  $\pm$  standard deviation being  $85.4 \pm 6.7$  years) included 19% having had a prior diagnosis of diabetes and the nearest infective process to osteomyelitis was cellulitis in 6%. Procalcitonin was found to be associated with infection at a level of 0.5ng/ml by Stucker *et al* (2007) but not independently of other variables including other markers used in this thesis research. Delévaux *et al* (2003) had previously expressed this figure as the level at which bacterial infection could be differentiated from other inflammatory processes. Again this was for hospitalized patients. Delévaux *et al* (2003) admit this work was for those with systemic bacterial

infections and suggest that the level of Procalcitonin may not rise in localized bacterial infections but no definition of 'localized' is given so it is not clear where osteomyelitis fits within this grouping. This appears to reinforce the findings of Reinhart, Karzai and Meisner (2000) suggesting only 'severe' infections cause Procalcitonin to rise.

An assessment of the clinical utility and limitations of Procalcitonin by Becker, Snider and Nylen (2008) has shown the degree of rise in levels that may be expected by a bacterial infection being the induction agent. The localised nature of osteomyelitis means that the results of this study were not of any use for comparison purposes.

Procalcitonin levels prior to the work of Uzun *et al* (2007) and Jeandrot *et al* (2008) had been noted to rise only during severe infections with systemic manifestations. It should be noted that it has not been considered a useful marker, when used alone, of infection as such as the large rise does not occur in local infections. However the work of Uzun *et al (ibid)* and Jeandrot *et al (ibid)* has shown some potential use in the recognition of infection in diabetic foot ulcers when used with other markers. Both of these studies were however undertaken on patients that had been admitted to hospital as a direct consequence of the infected foot ulcer. Diabetes related foot infections do not always present with the expected systemic signs (Caragee *et al* 1997 and Lewis *et al* 1998, Jeffcoate and Game 2006) presenting questions as to its usefulness alone.

The meta analysis by Simon *et al* (2004) of both Procalcitonin and C Reactive Protein as markers in sepsis in hospitalized patients has shown Procalcitonin to be superior to C Reactive Protein in the recognition of bacterial infection as opposed to viral infection. This agrees with the work using C Reactive Protein in paediatric cases by Gendrel *et al* (1999). The application of this work by Gendrel *et al (ibid)* to this study is not clear as it is not in paediatric patients and bone infection is generally bacterial in origin.



Uzun *et al* (2007) examined the utility of Procalcitonin in patients with clinically diagnostic signs of infection, including presence of pus, plus two of redness, warmth, swelling and pain. As discussed in the background these symptoms can be absent in diabetes making the diagnosis more complicated. The Uzun (*ibid*) study population was small, 27 with a diagnosed diabetic foot infection and 22 with a non infected diabetic foot lesion (by their diagnostic criteria mentioned above). As seen in other papers again the individuals had been admitted to hospital as a result of their foot problem. The results suggest Procalcitonin of all the markers had the highest area under the curve and greatest statistical significance for association with infection. Seven of the twenty seven with a recognized foot infection were diagnosed as having osteomyelitis (by the probe to bone test) but these were not analysed separately.

The recent pilot study by Jeandrot *et al* (2008) has suggested that Procalcitonin together with C Reactive Protein may be useful in the recognition of infection in diabetes foot ulcers. The aim of this Jeandrot *et al* (*ibid*) pilot study was to assess the diagnostic accuracy of inflammatory blood markers as an aid to making a distinction between non - infected ulcers (or those colonized with normal flora) and ulcers infected with virulent bacteria to determine the need for antibiotic therapy. It was a well conducted prospective study with reasonable numbers at the beginning (120 patients with diabetes but no foot ulcer and 153 with a diabetes related foot ulcer) however after the exclusion of individuals who had antibiotics within the previous six months this only left 45 individuals. It would be unusual in clinical practice to have an individual antibiotic free for this period of time within the natural history of a foot ulcer infection.

Jeandrot *et al* (2008) suggests a combination of C Reactive Protein and Procalcitonin derives, from a logistic regression with other markers, an Area Under the Curve result of 0.947. This was in comparison with other Receiver Operating Characteristic curves (the other markers included orosomcoid, haptoglobin, white cell count and Neutrophil Count, the first two not being readily available from most hospital laboratories). This study was performed in order to determine if early infection could be differentiated from bacterial colonisation. As many of the other previous studies

with inflammatory blood markers this was performed on hospitalized patients. However its suggested use in combination with C Reactive Protein led to its inclusion in this study.

In the assay method used in this research it is stated that Haemoglobin (up to 500mg / dl), Albumin (up to 1g / dl) and Triglyceride (up to 1g/ dl) have no 'interference'. Other 'interfering substances' that may require consideration that it is not known whether they were present in the sample individuals include Furosemide (a loop diuretic drug) at levels of 2mg/dl and several antibiotics that it was unlikely the individuals were taking including Imipenen (at 1.18mg/ml), Vancomycin (at 3.5mg/ml) and Cefotaxime (at 90mg/dl).

Combining Procalcitonin, white blood cell count and C Reactive Protein has been shown to be beneficial particularly to diagnose infection Bell *et al* (2003), Castelli *et al* (2004), Thayyil *et al* (2005) and Kofoed *et al* (2007). All these four studies show levels of all three markers to be raised in bacterial infections as opposed to other infections or non infectious causes of inflammation. However none of these papers are directly applicable to the individuals in this study. The papers with the exception of that by Thayyil *et al (ibid)* are studies of adults in Intensive Care settings. That of Thayyil *et al (ibid)* being based upon paediatric patients with no obvious focus of infection.

The non specific markers of inflammation which may have some use appeared to be multiple. As non specific markers of inflammation i.e. not able to distinguish inflammation from infection the use of multiple markers seemed prudent.

## 2.8 Proposed studies using inflammatory blood markers

The final choice of markers was determined having examined the literature for those that had shown some possible use before; the use of inflammatory blood markers in an out patient setting is novel as is the proposed series of markers. Using a combination of short term markers (less than 24 hours), e.g. C Reactive Protein, and long term, e.g. Plasma Viscosity, allows a full coverage of the disease process. The

confusion over the affects of markers would suggest the need for the use of multiple markers and is confirmed by Sattar (2006a). The reasoning behind this is again the ineffective immunological response to pathogens. Eneroth, Larsson and Apelqvist (1999) developed levels of Haemoglobin, Erythrocyte Sedimentation Rate, C Reactive Protein and White Blood Cell count that they considered typical of osteomyelitis only, deep soft tissue infection only and combined infection that maybe useful as a control to measure this research against.

As non specific markers of inflammation a determination of a baseline level to recognise infection was found to be lacking in previous research.

The difficulty with previous studies not defining the confounders e.g. cardiovascular disease and neuropathy has meant that there is a difficulty in being able to compare this work to previous work. The use definitions used of cardiovascular and neuropathy here are at the beginning of this thesis.

The studies in this thesis were conducted in an attempt to find alternative methods of diagnosing osteomyelitis. The enquiry using inflammatory blood markers was conducted as the only work that could be found prior to this study was based upon patients that were so acutely unwell that they were in – patients within the hospital setting. It was hoped that a level of a single marker, or a group of markers would be found that would be suggestive enough to make osteomyelitis very probable.

The final choice of markers was Neutrophil count, Haemoglobin, C Reactive Protein, Plasma Viscosity and Procalcitonin.

Individuals were assessed as having a clean ulcer – no sign of infection as determined by clinical signs and symptoms despite the acknowledged problems that this has in individuals with diabetes, a cutaneous infection by a similar diagnostic strategy, suspected osteomyelitis determined by depth of wound and /or probing

bone and confirmed osteomyelitis by a probe to bone test being positive, plain X ray indicating bone destruction, MRI, SPECT/CT or bone biopsy.

## 2.9 Proposed studies using SPECT/CT

The investigation using the alternative scanning technique of SPECT/CT was carried out in an attempt to determine if this form of imaging is of comparative use to MRI. Some individuals are unable to undergo the MRI procedure and an alternative that gives as much detailed information would be very welcome. To determine comparability of the imaging techniques subjects in this part of the study had both forms of imaging with blind reading of the results by the relevant specialist radiologist.

## 2.10 The study and wound classification

The study also needed to attempt to recognise the ulcer from a classification view point to see if there was any link between results and ulcer classification that was quickly and simply available in a clinical setting.

### 2.10.1 Ulcer classification systems

In an attempt to correlate the stage of ulceration with the blood results two foot ulcer classification systems were adopted. Classifications enable selection of populations of similar lesions for prospective research (Macfarlane and Jeffcoate (1999). This means it is necessary to give precise descriptions, including the presence or absence of infection and vascular compromise (Lavery, Armstrong and Harkless 1996). Good classifications encourage clarity of thought and promote better understanding of disease processes, which in turn lead to better management (Jeffcoate, Macfarlane

and Fletcher 1993). They also should be simple enough to be remembered, and yet precise enough to be useful (*ibid*). The one system used was that known by the abbreviations of its components the S(AD) SAD was used. This system grades the ulcer according to the variables of size – in both area and depth, the presence of sepsis, degree of arteriopathy (circulatory disease), and degree of denervation (nerve damage). These are the elements that contribute to that nature of the lesion. Each of the five categories is ranked on a four-point scale (0, 1, 2, 3). It is the only such classification with a process of validation against a wide variety of end-points to ensure its reliability is this S(AD) SAD system (Treece *et al* 2004). The clinical measurement methods of assessing ischaemia and neuropathy are recognised by the original authors as being imprecise but recognise typical clinical practice as opposed to research and are acknowledged as a possible downfall in this classification. The result is an arrangement simple enough to be universally understood and applied to a large number of lesions in routine practice, and yet, is precise enough to be meaningful (Jeffcoate and Game 2006). Macfarlane and Jeffcoate described the original classification in 1999. This system has distinct advantages over the other most frequently used classification, the Wagner Grading System. Wagner was meant to classify lesions and not infections (Eneroth, Larson and Apelqvist 1999). As a result it fails to address the localization of infection, the systemic manifestations, the vascular status and host factors in diabetic foot lesions (LeFrock and Joseph 1995); this is also true of the University of Texas San Antonio Diabetic Wound Classification System (Lavery, Armstrong and Harkless 1996). As a successful classification it needs to be, and indeed is, based on all the key elements that contribute to the nature of the lesion (Macfarlane and Jeffcoate 1999).

The key elements of the classification need to be subdivided according to the extent to which they contribute to the status of the lesion. It is accepted that this will to some extent be empirical as the sub divisions maybe based on either quantitative or qualitative measures. Each of the components is explained with its levels of assessment. The categories do not follow a logical progression or even increasing severity but they are mutually exclusive and, individually, they are applicable to the vast majority of lesions seen in clinical practice.

In the S(AD) SAD classification of the severity of ischaemia is used when the ischaemia is not critical. As for infection the order of increasing score in the assessment tool is consistent with increasingly reduced likelihood of healing.

Also included in the S(AD) SAD assessment tool are wound surface area and sepsis. Area is recognised by Macfarlane and Jeffcoate (1999) as being an important factor. They also advocate that in prospective research the measurement should be accurate and promote the use of sterile transparent sheets marked with a grid. In the S(AD) SAD classification system the gradation is skin intact, area  $< 1 \text{ cm}^2$ ,  $1-3 \text{ cm}^2$ ,  $> 3 \text{ cm}^2$ . Depth has been acknowledged as a risk factor for amputation (Armstrong, Lavery and Harkless 1998) and as such is an essential requirement in a classification system. Following the work of Lavery, Armstrong and Harkless (1996), validated by Armstrong, Lavery and Harkless (1998), the ranking system is skin intact, either non-ulcerated or healed, superficial, involving skin and subcutaneous tissue but not tendon, periosteum, or joint capsule, penetrating to tendon, periosteum, or joint capsule, involving bone or joint spaces.

Inefficient Neutrophil bacteriocidal mechanisms have been recognised for a long time as partially responsible for the increased susceptibility of patients with diabetes to infection (Tan *et al* 1975). Sepsis can be difficult to both diagnose and categorize. Macfarlane and Jeffcoate (1999) suggest that the diagnosis is primarily clinical but recognise the confounding of the problem by other diabetes complications, notably ischaemia and denervation. The sub division of sepsis is problematic as the categories are discontinuous and reflect qualitatively different types of infection. The division used in the S(AD) SAD system does have some conformity of increasingly poor prognosis. It is recognised that the cumulative effect of features such as depth and infection contribute to the increased likelihood of amputation (Armstrong, Lavery and Harkless 1998).

The Internal Working Group on the Diabetic Foot had developed the other classification system referred to as the PEDIS system (Apelqvist *et al* 1999). This is an abbreviation of the five categories assessed in the system – namely Perfusion, Extent/size, Depth/tissue loss, Infection and Sensation. These categories are, similarly to the SAD S(AD) system, clinically assessed with grading within perfusion, depth, infection and sensation. It is recognised that this system has yet to be validated formally. The categories and grades were defined on the basis of their relevance for research by internationally recognised experts (Schaper 2004) where this it is argued provides face validity. It is worth repeating the reproducibility in terms of intra – and inter – observer variability has yet to be evaluated (*ibid*).

What is known to contribute to this syndrome of ‘the diabetic foot’ but is not included in either assessment tool, or many of the others available, are issues such as uncontrolled hyperglycaemia, limited joint mobility and structural foot deformities.

No assessment tool has yet been developed and validated that includes all the variables that can add to this syndrome complicated further by the fact many of the factors can not be quantified.

The use of ulcer classification systems such as the S(AD) SAD and PEDIS as described however allowed recognition of the prime cause of the ulceration which was useful in the results section where the determination of the effect of neuropathy could be considered (see Chapter 5).

The burden of diabetic foot disease is set to increase in the future since by the reckoning of Boulton *et al* (2005) as the contributory factors to foot disease, such as peripheral neuropathy and vascular disease are present in more than 10% of people diagnosed with type 2 diabetes (UKPDS 33) and it has been recognised by New *et al* (1998) the first year after diagnosis is a ‘period of danger for foot ulcers and amputations’.

The complex nature of the disease process including the diagnosis and provision of appropriate treatment can be seen to be challenging.

This chapter has attempted to show the context in which this research was considered to be worthwhile showing what has been found to date and the gaps in the knowledge we currently have about diabetic foot infections.

The following chapters will consider the research that was undertaken by describing the methodology, results and discussing the results. The discussion to previous work is limited by the lack of comparable work.



## **Chapter 3 Methodology The use of inflammatory blood markers in the diagnosis of osteomyelitis**

This chapter will outline the approach used in the research study that examined the potential use of inflammatory blood markers in the diagnosis of osteomyelitis. The need for these tests to have sufficiently high specificity and sensitivity in the diagnosis is not discussed at this point in time but in Chapter 5.

### **3.1 Research question**

Do inflammatory blood markers have a role to play in the early diagnosis of bone infection in people with diabetes associated foot ulcers in an out patient setting?

### **3.2 Aim**

To ascertain if a level of the chosen inflammatory blood marker(s) is useful for the early diagnosis of osteomyelitis in the diabetic foot.

### **3.3 Research objectives**

To establish the ‘normal’ range of each marker in the diabetes population with

1. A clean, non-infected, foot ulcer
2. A skin infection associated with a foot ulcer
3. An infected foot ulcer and suspected osteomyelitis
4. An infected foot ulcer and proven osteomyelitis and
5. To compare the range of values of each marker in each sub group, and with the accepted ‘normal’ range.

Individuals were placed within these groups by clinical signs and symptoms plus, where appropriate, diagnostic imaging.

A clean, non-infected, foot ulcer was diagnosed by the absence of any of the following: pus, heat, swelling, pain and obvious inflammation (Lipsky *et al* 2004). The wound was shallow with healthy granulation tissue evident.

Infection was diagnosed clinically by the presence of purulent secretions or at least two of the cardinal manifestations of inflammation as described above i.e. redness, warmth, swelling or induration, and pain or tenderness (*ibid*). Differentiation between skin, cutaneous, infection, and osteomyelitis was made on the clinical grounds of a 'positive probe to bone test' that led to further imaging for confirmation (or to be able to discount) osteomyelitis. The simplest form of imaging being a plain X ray. If this was clearly diagnostic of osteomyelitis no further imaging examination was considered. If the X ray was inconclusive an MRI was ordered and if the patient was willing to undergo the imaging part of the research, a SPECT/CT was also booked. A non-healing ulcer at 4-6 weeks with suitable debridement and off loading without any other obvious cause was also suspected to have osteomyelitis and was investigated as described above in terms of imaging.

Confirmed osteomyelitis was considered when a plain X ray was unmistakably showing bone destruction, or when either MRI or SPECT/CT was used and was diagnostic.

The groups were not mutually exclusive in that an individual with a cutaneous infection may also have had osteomyelitis.

### 3.4 Protocol

The following conditions have been defined at the beginning of this document and this is how they are referred to within this work;

- Anaemia;
- Cardiovascular disease;
- estimated Glomerular Filtration Rate (eGFR)
- Neuropathy
- Neutrophil
- Peripheral Vascular Disease;
- Reduced renal function.

### 3.5 Summary

This study investigates the use of inflammatory blood markers in making a diagnosis of osteomyelitis in foot ulcers associated with diabetes.

### 3.6 Keywords

Inflammatory blood marker Neutrophil Procalcitonin Haemoglobin Plasma Viscosity C Reactive Protein Diagnosis Osteomyelitis

### 3.7 Confounding factors

The use of antibiotics, either oral or intravenous, is not discussed in any previous papers as a potential source of reduction in the inflammatory response. In this piece of research their use was noted but not the duration of use and/or if the infection was acute or ongoing being contained by the use of the anti-microbial agent. Nor was the causative micro organism typed as it has been suggested that some may be more virulent than others and as such cause a response of different magnitude (Armstrong *et al* 1996b). In retrospect these may have had an impact worthy of consideration and should have had data collected about them. The taking of superficial wound swabs is not part of the usual practice in Bath due to this type of swab often giving little useful information. Very few individuals would have had a deep wound swab taken. The degree of inflammatory change may be related to the degree of inflammation

related to the acute or chronic nature of the wound and the virulence of any causative infecting organism.

Cardiovascular disease was defined in this study, in the absence of any guidelines as to what other studies have used, by the use of medications associated with cardiovascular disease. These included statins, fibrates, anti-hypertensives (very broadly as such including thiazides and calcium channel blockers amongst others), anti - platelet drugs and anti-coagulants.

### 3.7.1 Recognition of confounding factors

The assessment and data gathering tool was designed to collect information about the potential confounding issues present at time of consent as recognised from the previous papers on the subject. This included renal function measurements, the use of different medications used to treat diabetes including Insulin and Metformin, history of both cardio- and peripheral vascular disease and both type and duration of diabetes as a disease, and the presence of neuropathy.

### 3.7.2 Managing confounding factors

The database set up to collate the data included information on the potential confounding factors. This allowed for the analysis to potentially consider the effects that each may have on the results. The problems of non specific use of terminology have been discussed before and the problem it presents in comparing research.

The use of multiple markers reduced the potential for an error due to confounding if only one marker had been used. The different processes by which the markers are 'activated' by the inflammatory process and with different potential confounding factors affecting different markers it was felt the result would be more robust. The markers used were C Reactive Protein, Haemoglobin, Neutrophil Count, Plasma Viscosity and Procalcitonin. Procalcitonin was added part way through the study

after the publication of a paper that had suggested its potential usefulness (Jeandrot *et al* 2008) in combination with C Reactive Protein in the recognition of infection in diabetic foot ulcers. (An amendment to the protocol was sent to the ethics committees involved and approval given before it was included.)

### 3.8 Multiple versus single markers

This confusion over the effect of disease on markers would suggest the need for the use of multiple markers and is confirmed by Sattar (2006). The reasoning behind this is, again, the time differential in producing markers, the effect of diabetes as a disease process and possibly an ineffective immunological response to pathogens. Eneroth, Larsson and Apelqvist (1999) developed levels of Haemoglobin, Erythrocyte Sedimentation Rate, C Reactive Protein and White Blood Cell count that they considered typical of osteomyelitis only, deep soft tissue infection only, and combined infection. This was considered potentially useful as a control to measure this research against for Haemoglobin and C Reactive Protein.

No work appears to have looked at the utility of inflammatory blood markers in the out - patients setting exclusively.

### 3.9 Setting of research studies

The setting of the research was a multidisciplinary Diabetes Foot Clinic held in an English District General Hospital where the researcher was the lead podiatrist. As such the setting was an out patient setting where individuals had been referred for help from the specialist team with 'difficult' or 'non responding to treatment' ulcers. The hospital has 687 beds and covers a population of some 550000 from mixed urban and rural communities. It is supported by community hospitals with Minor Injury Units. Referrals to the clinic came from community podiatrists, practice and district nurses and General Practitioners. Many of the individuals had marked co-

morbidities including a history of severe foot problems. Severe in this instance meaning a previous need for intra venous antibiotics and / or surgical debridement.

### 3.10 Sample

Individuals were approached that attended the multiprofessional Diabetes Foot Clinic in an acute district general hospital. The individuals had been referred for specialist care advice and treatment of their foot ulcer to the diabetes team by community podiatrists, practice and district nurses and General Practitioners. The Diabetes Foot Clinic team consist of a consultant diabetologist with a special interest in feet, a consultant vascular surgeon with a special interest in diabetes and feet, two experienced podiatrists, an orthotist all with rapid access to a consultant microbiologist with a special interest in infectious diseases, consultant dermatologists, consultants in orthopaedics, vascular technicians and plaster technicians.

The use of broad non specific criteria for the recruitment process was an attempt to recruit as many individuals as possible without affecting clinical care. These may be the individuals who could be seen to be requiring immediate emergency care for the presenting problem. It was ensured it could be seen there was no coercion into partaking. Those with a disease different to diabetes that was likely to affect the inflammatory markers were not approached nor those who had the potential to be harmed by the process e.g. the exposure to an unborn child of the radiation in the Single Photon Computed Tomography / Computed Tomography part of the study.

### 3.11 Method; Ethical statement

Approval from the relevant NHS trusts and from both the University of Bath and COREC ethics system was sought and followed. This ensured that ‘good clinical practice’ was maintained throughout, and either taking part or refusal did not compromise clinical care for that individual or any others. Research governance

procedures including securing Research and Development (R&D) approval was also performed. The storage of patient sensitive information was also on a NHS computer that was sign – on and password protected with additional passwords to access any research material.

### 3.12 Method; Ethical considerations

The additional treatment over and above standard care received in the clinic involved the individual undertaking venepuncture where this was not routine practice. It is estimated this added approximately five minutes to the clinic attendance time with the potential to cause minor inconvenience and minor discomfort with the chance of a needlestick injury being rare.

The confidentiality required in obtaining the participants personal information from the diabetes data base and laboratory system were covered in the course of normal clinical practice by the Caldicott Guardian and Data Protection principals. This means only the data that was required for the study was accessed and stored in a manner to keep it safe.

Any unexpectedly abnormal blood results were referred to either the patient's General Practitioner or a hospital specialist as agreed in the written consent.

Patients attending for a plain X ray were sent to the main X ray department where a 'sit and wait' system applies for individuals undergoing examination. No preferential treatment was given to speed up the process.

All information was stored on a NHS computer user name and password protected. The results of the blood analysis and imaging techniques were accessed via electronic systems further user name and password protected.

The data was anonymised within the results database.

### 3.13 Inclusion / exclusion criteria

#### INCLUSION

- to have a diabetes related foot ulcer
- to be able to give informed consent

#### EXCLUSION

- to have overwhelming sepsis requiring immediate medical and/or surgical treatment
- to be unable to give informed consent
- to have an estimated Glomerular Filtration Rate of less than 60ml/hour
- to have a concurrent disease likely to affect inflammatory markers e.g. rheumatoid arthritis
- to be pregnant
- to be breast feeding.

### 3.14 Consent

Individuals were given the patient information sheet as they reported to reception in the Diabetes and Endocrinology centre to allow for some time to read the information prior to being approached to take part in the research. The patient information leaflet had been accepted by the ethics committee as having the wording of a certain size and font to assist legibility and also in language suitable for lay individuals.

The care that the individual was booked into the clinic to receive was provided prior to any discussion about the research.

The patient information sheet asked individuals to consider giving their consent for the researcher for both the research, i.e. venepuncture immediately and referral for



imaging where deemed appropriate, and, also in addition to use the diabetes database to determine basic demographic details and some baseline medical details stored about them. The diabetes database is 'sign on' and password protected. The details noted were age, gender, duration of diabetes, type of diabetes. The laboratory results system that was used to gain access to information such as renal function is also 'sign on' and password protected. (This was also the system for the return of the results of the venepuncture for the markers in the research.) Such co-morbid factors that required consideration included renal function and history of ischaemic heart disease.

After the provision of clinical care the individual was approached by the researcher to ask if they had had the opportunity to consider the information provided. Two individuals admitted to not being able to read sufficiently well to comprehend the sheet. A brief summary was provided by the researcher with the offer of an independent individual (the clinic nurse) reading the sheet to them to allow an informed choice as to taking part. Both agreed to this and subsequently consented.

Routine care was continued to be provided for all patients – those who choose to participate and those who did not. This included the use of antibiotic therapy and MRI scans where appropriate.

Any questions that were asked were answered by the researcher. Consent was thus informed. Written consent was obtained from all patients willing to participate. It was also made clear at this point that the individual may be asked to participate again should there be a significant change in the presentation of their ulcer.

### 3.15 Method; Wound assessment

To ensure comparisons of similar ulcers occurred they were classified using the two ulcer classification systems; the S(AD) SAD and PEDIS ulcer assessment systems.

The application of the tool was followed as described in the original paper (Macfarlane and Jeffcoate 1999) with the exception of assessment of neuropathy and this is detailed within the definitions section of this thesis and below.

The measurements of; S size was measured using a sterile plastic film with a grid that can be placed against the wound to determine area (A), observation of depth used observation of tissue seen together with a sterile wound measuring probe (D), observation of tissue damage for (s)epsis was determined by the presence or absence of inflammation and the presence of exudate S, palpation of, or use of a hand held Döppler to evaluate pedal pulses (posterior tibial and dorsalis pedis) was performed by an experienced clinician to determine (a)rterial disease A, and the assessment of (n)erve damage (denervation) by the presence/reduction/ absence of pin prick sensation on the dorsum of the affected foot using a monofilament D. This it is acknowledged is different from the original research on this tool. The original used a Neurotip™, a disposable sharp plastic instrument for testing for the lack of sensation, which is now considered to be better tested using the monofilament as harm is less likely.

The clinical measurement methods of assessing ischaemia and neuropathy are imprecise but recognise typical clinical practice as opposed to a research model and are acknowledged as a possible weakness in this classification.

Each of the components of the wound is graded from 0 to 3. The resulting score is simple enough to be applied to a large number of lesions in routine practice, and yet, is precise enough to be meaningful (Jeffcoate and Game 2006).

The PEDIS approach uses clinical assessments of the individual components and this was followed as in the original work described by Apelqvist *et al* (1999).

A comparison of the two systems chosen was made to determine if either is useful either alone or in combination with the blood tests to establish a diagnosis of osteomyelitis.

The ulcer classifications were performed on a data gathering pro-forma that was kept securely with a copy of the patients consent in a locked filing cabinet. The original consent was filed in their medical notes and a third copy given to them to keep.

### 3.16 Method; venepuncture

The standard technique for taking venous blood using a tourniquet on the upper arm to distend the brachial and cephalic veins was used. The method used was the Vacutainer® manufactured by Becton Dickinson pre-analytical diagnostics. A total of two samples were taken; one for the full blood count (including Haemoglobin level and Neutrophil Count) and C Reactive Protein level and one for Procalcitonin and the Plasma Viscosity. This last sample was required to be both taken and used immediately for the Plasma Viscosity and then spun and stored separately in a freezer. This was because the samples for Procalcitonin analysis were sent away for analysis as a batch. This meant a total of 9 mls of venous blood in two samples was taken at or near as possible to the date of consent.

The Becton Dickinson blood collection system is a closed evacuated system, which consists of a double-ended needle with safety valve, Becton Dickinson Vacutainer® holder and sterile Becton Dickinson Vacutainer® tubes with pre-measured vacuum. The collection tubes are made from medical grade Polyethylene Terephthalate which is clear, shatter resistant and safer clinical alternative to glass. Blood is collected by screwing the sleeve-covered end of the needle into the holder, then puncturing the patients' vein with the other end. After performing venepuncture the tube is then pushed down into the holder, and the pre-measured vacuum of the tube allows the volume of blood to be drawn. The tubes are colour coded according to the appropriate additive and international standards (ISO 6710).

The advantages to this system include;

Blood being drawn directly into the tube limits user exposure and

The method of blood collection is standardized including the amount of blood mixing it with a consistent quality and quantity of additive.

The tubes used were SST (the gold top) which contains a polymer gel and clot activator that accelerate the clotting process and EDTA (the lavender top). The additives are specific to the tests required. They were required to be drawn in that order i.e. coagulation followed by other additives. The SST tube upon centrifuging allows movement of the gel to the upper part of the tube forming a barrier between the top layer of serum and bottom layer of cells and fibrin. The gel allows easy separation of the two parts. The EDTA tube inhibits coagulation by eliminating the calcium in the blood reducing the platelet activation when the blood comes into contact with the inner surface of the tube.

The C Reactive Protein, Haemoglobin level, Neutrophil Count and Plasma Viscosity were measured immediately within the Royal United Hospital, Bath pathology laboratory. As the Procalcitonin could not be processed on site after the Plasma Viscosity measurement the storage of the remainder of the SST tube was within the hospital laboratory freezer at -20°C. This storage was with access being limited to the researcher and one of the consultant clinical biochemists. The Instruction Manual from BRAHMS indicates that samples may be frozen and thawed three times (BRAHMS PCT LIA version 05us).

For those individuals without clinical suspicion of osteomyelitis (or those in whom osteomyelitis was confirmed by plain X ray) this was all the blood taken for this episode.

Should any of the ulcers have changed during the study period whilst the individual was under follow up they were asked to consider consenting to the entire process again.

The venepuncture of the group undergoing imaging studies was at the consent and clinical suspicion phase and repeated at a time as close as possible to the

confirmation/exclusion of bone infection by the MRI scanning procedure and if necessary and consented to SPECT/CT. To ensure this correlation between disease process and blood markers venepuncture was undertaken within a week of the MRI and SPECT/CT scans.

### 3.17 Data collection tool

The data collection tool was developed to allow all the information for each participant to be on one piece of paper and as such included date of consent, date of venepuncture, results of venepuncture, referral (or not) to imaging with second venepuncture results, S(AD) SAD ulcer score, age, gender, type of diabetes (1 or 2) , duration of disease, use of potential confounding medication to blood results – i.e. Insulin, Metformin hydrochloride, and history of renal impairment, cardiovascular and peripheral vascular disease. The completed forms were stored in a locked filing cabinet. The tool was amended as the protocol changed to include Procalcitonin.

### 3.18 Method; Imaging studies

Any wound with suspicion of osteomyelitis was sent for plain X ray as a baseline. Any individual with continued suspicion despite the result (made by the radiology team reading the film not just the clinicians in the Diabetic Foot Clinic looking at the film on the computer immediately after imaging) was referred for Magnetic Resonance Imaging (where appropriate both clinically and individually) and Single Photon Computed Tomography / Computed Tomography. If confirmed, the method used was noted in the results database.

For more detail about the scanning methodology see chapter 6.

### 3.19 Analysis; of the blood markers

Data was returned from the pathology laboratory via an electronic system. This required the clinician collecting the data to have a 'sign on' name and password. An audit trail is produced whenever an individual 'logs on' and as such it can be ensured

that only relevant information is retrieved. Each individual will be identified by their unique hospital number to access the results. Access to the hospital appointment system to look up personal information from the number is also 'sign on' and password protected. Data was stored on a non networked PC with password access to open and also to change the document in terms of adding more results.

### 3.20 Measurement of the specific inflammatory blood markers

#### 3.20.1 Haemoglobin and Neutrophil Count

The measurement of Haemoglobin levels to determine the presence or absence of anaemia was performed on a Beckman Coulter LH750 automated analyser. This was also used for the determination of the Neutrophil Count. The quality control within the laboratory meant any grossly abnormal reading was checked 'manually' using a blood film and microscopy.

#### 3.20.2 Plasma Viscosity

The measurement of Plasma Viscosity was performed on an automated Benson Viscometer with an in built quality control solution to ensure accuracy.

#### 3.20.3 C Reactive Protein

The measurement of C Reactive Protein was analyzed on a Roche analyzer based upon a particle-enhanced immunturbidimetric assay – the immunological test principle. This involves the use of a buffer to produce an anti - C Reactive Protein antibody which is then coupled with latex microparticles. The level of the antibody/antigen complex can be determined turbidimetrically after agglutination and this is what gives the measurement.

#### 3.20.4 Procalcitonin

The measurement of Procalcitonin was using the B.R.A.H.M.S. Immunoluminometric assay. This method detects Procalcitonin at a very low level and is useful in the detection of bacterial infections. The method involves Procalcitonin acting as an antigen binding with two antigen-specific antibodies (two different sites exist). One of the complexes formed (the antibody acting as a tracer) is luminescence labelled and the other is fixed to the inner walls of the tubes. During the incubation of the sample both antigen-antibody complexes combine within the sample to form 'sandwich complexes'. This allows the total amount of Procalcitonin is measured by an estimation of the luminescence.

## **Chapter 4 Results The use of inflammatory blood markers in the diagnosis of osteomyelitis**

### **4.1 Statistical analysis of results of blood samples**

The data was collated into a database specifically designed for the study and coded to allow for easy analysis of each of the markers and each of the groups of infection. The computer software package of the Statistical Package for Social Scientists (SPSS) version 14 was used.

The following chapter provides the results of the analysis of the blood samples. The analysis includes a calculation of mean (and / or median) value of each marker overall and in each of the groups; clean, cutaneous infection, suspected osteomyelitis and conformed osteomyelitis. NB these groups are not mutually exclusive in that an individual may have a cutaneous infection in addition to a confirmed osteomyelitis. A Receiver Operator Characteristic curve was produced for each group and a regression analysis for each group. The potential confounders were also considered within regression analysis. Correlation between the inflammatory markers was explored to determine if there was any relationship between the different markers being used.

A total of three individuals agreed and signed the consent but did not agree to have venepuncture whilst in clinic and did not go on to have blood taken. One asked at a latter date for the blood results to be withdrawn from the study and this wish was fulfilled.



## 4.2 Sample characteristics - age

Descriptive statistics; age, minimum 35 years, maximum 93 years, mean 66 years (standard deviation  $\pm$  14 years).

Table 4.1 sample characteristics - gender

<b>Gender</b>	<b>Frequency n (%)</b>
<b>Male</b>	70 (78%)
<b>Female</b>	20 (22%)

Table 4.2 sample characteristics - type of diabetes

<b>type of diabetes</b>	<b>Frequency N (%)</b>
<b>type 1</b>	17 (19%)
<b>type 2</b>	73 (81%)

Table 4.3 sample characteristics – diabetes treatment

<b>Treatment</b>	<b>type 1 (n=17) (%)</b>	<b>type 2 (n=73) (%)</b>
<b>Insulin</b>	17 (100%)	38 (52%)
<b>Metformin</b>	2 (12%)	41 (56%)

## 4.3 HbA1c levels

(n=79) Mean 8.0% minimum 5.2% maximum 14.9%

Table 4.4 sample characteristics – duration since diagnosis

<b>Duration</b>	<b>Years</b>
<b>Minimum, maximum</b>	1.00, 55.00
<b>Median years, interquartile range</b>	13.50, 5.00 – 20.25

Numbers with clean wounds / with cutaneous infection / with suspected osteomyelitis and with confirmed osteomyelitis (NB groups are not mutually exclusive i.e. an individual may have a cutaneous infection and also have confirmed osteomyelitis).

The use of the ulcer classification systems did not add to the diagnostic prediction of wound types either alone or in combination with the blood results but did allow for comparison of similar wounds to occur within the analysis.

Table 4.5 numbers of wound types

Wound type	yes (%)	no (%)
Clean	33 (37)	57 (63)
Cutaneous infection	61 (68)	29 (32)
Suspected osteomyelitis	32 (36)	58 (64)
Confirmed osteomyelitis	33 (37)	57 (63)

Table 4.6 descriptive statistics in all wound types for each inflammatory marker

Marker	Accepted normal range	Number	Minimum	Maximum	Mean (±Std dev)	Median (IQR)
Neutrophil count x 10 <sup>9</sup> / l	2 – 7.5	90	1.9	16.0	6.07 (±2.67)	5.60 (4.08 – 7.40)
Haemoglobin g /dl	>11	90	8.4	15.50	12.30 (±1.61)	12.40 (11.18 – 13.40)
Haemoglobin g /dl excluding potential renal anaemia	>11	83	8.6	15.10	12.38 (±1.48)	12.40 (11.40 – 13.40)
C Reactive Protein g / l	<5	90	4.99	215.00	22.95 (±33.37)	10.00 (4.99 – 27.00)
Plasma Viscosity mpas	1.50 – 1.72	89	1.50	2.72	1.83 (±0.21)	1.77 (1.77 – 1.91)
Procalcitonin ng / ml	<0.3	49	0.01	0.93	0.06 (±0.14)	0.03 (0.02 – 0.04)

### 4.3 Summary statistics for each marker in each state separately

The figures in Appendix 9 are descriptive for each marker and each wound condition with a summary table overleaf. Summary descriptive statistics are corrected to two decimal places except for Procalcitonin where the low figure has meant three

decimal places are used. (The individuals with a result  $\geq 0.6$  ng/ml have been removed see page 103).

Those individuals with a reduced renal function, such that anaemia maybe a consequence have been removed from all statistics where Haemoglobin is considered from here on.

Table 4.7 summary statistics of each inflammatory marker in each wound condition

n = number SD = standard deviation IQR = interquartile range

<b>Marker</b>	<b>Accepted Normal range</b>	<b>Clean wounds</b>	<b>Cutaneous infection</b>	<b>Suspected osteomyelitis</b>	<b>Confirmed osteomyelitis</b>
<b>Neutrophil count <math>\times 10^9</math> / l (mean)</b>	2 – 7.5	5.57	6.43	6.34	6.40
<b>Haemoglobin g / dl (mean)</b>	>11	n = 28 12.64 SD $\pm 1.54$	n = 57 12.29 SD $\pm 1.50$	n = 30 12.26 SD $\pm 1.57$	n = 29 12.24 SD $\pm 1.64$
<b>Haemoglobin g / dl (median)</b>	>11	n = 28 12.60 IQR 11.95,13.75	n = 57 12.30 IQR 11.3,13.2	n = 30 12.30 IQR 10.98,13.87	n = 29 12.40 IQR 10.90,13.80
<b>C reactive protein (mean) mg / l</b>	>5	n = 30 11.03 SD $\pm 15.89$	n = 61 28.16 SD $\pm 38.51$	n = 32 24.53 SD $\pm 40.65$	n = 33 29.33 SD $\pm 42.58$
<b>C reactive protein (median) mg / l</b>	>5	n = 30 6.00 IQR 4.99,10.5	n = 61 13.00 IQR 5.49,32.00	n = 32 10.50 IQR 4.99,24.25	n = 33 13.00 IQR 4.99,31.50
<b>Plasma viscosity (mean) mpas</b>	1.5 – 1.72	n = 30 1.76 SD $\pm 0.13$	n = 60 1.87 SD $\pm 0.22$	n = 31 1.83 SD $\pm 0.18$	n = 32 1.86 SD $\pm 0.22$
<b>Plasma viscosity (median) mpas</b>	1.5 – 1.72	n = 30 1.75 IQR 1.66,1.86	n = 60 1.79 IQR 1.70, 1.98	n = 31 1.81 IQR 1.70,1.93	n = 32 1.82 IQR 1.71,1.95
<b>Procalcitonin (mean) ng / ml</b>	<0.3	n = 17 0.027 SD $\pm 0.008$	n = 34 0.101 SD $\pm 0.225$	n = 22 0.035 SD $\pm 0.025$	n = 21 0.100 SD $\pm 0.210$
<b>Procalcitonin (median) ng / ml</b>	<0.3	n = 17 0.030 IQR 0.209, 0.300	n = 34 0.030 IQR 0.020,0.020	n = 22 0.030 IQR 0.020,0.040	n = 21 0.030 IQR 0.020,0.050

#### 4.4 Box plots of each marker in each wound state together

Haemoglobin for those with the potential for renal anaemia removed. Accepted normal ranges for Neutrophil Count, Haemoglobin, C Reactive Protein and Plasma Viscosity are for the Royal United Hospital, Bath, United Kingdom and for Procalcitonin for Queens University, Belfast, United Kingdom.

The term selected is used when a result has been selected as belonging to that state (that is either clean, with cutaneous infection, suspected or proven osteomyelitis). The extreme cases that do not fit within the range of the group are indicated by the circles. It can be seen some extreme cases appear over several markers but it is not consistently the same individual in all.

Figure 4.4.1 Comparison of Neutrophil Count in all wound conditions

Accepted normal  $2 - 7.5 \times 10^9 / l$

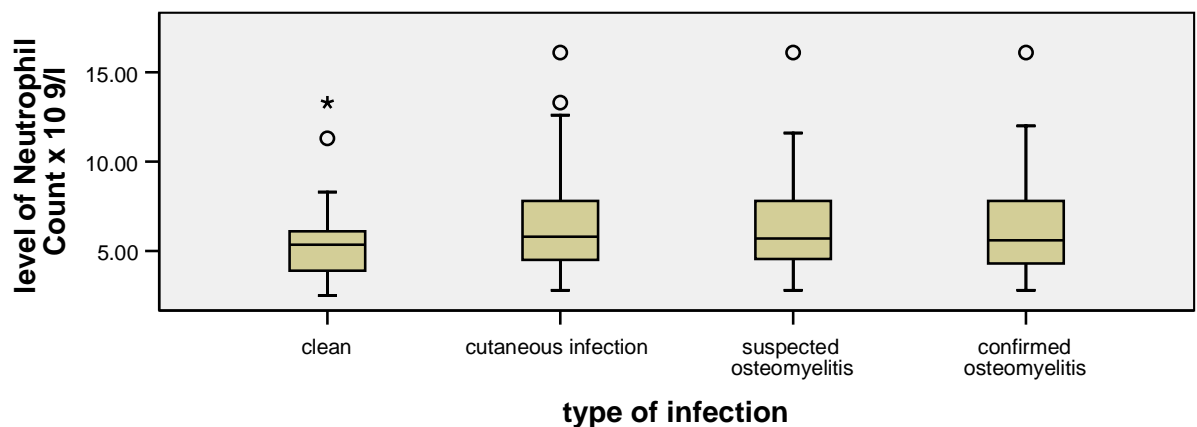


Figure 4.4.2 Comparison of Haemoglobin level in all wound conditions (excludes renal impairment)

Accepted normal ♂ 13.5 – 15.5 g/ dl ♀ 11.5 – 15.5 g/ dl

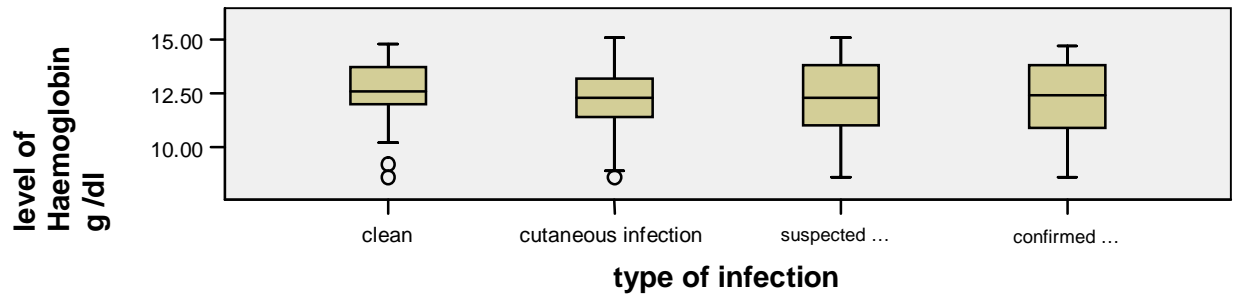


Figure 4.4.3 Comparison of CRP level in all wound condition

Accepted normal < 5 mg / l

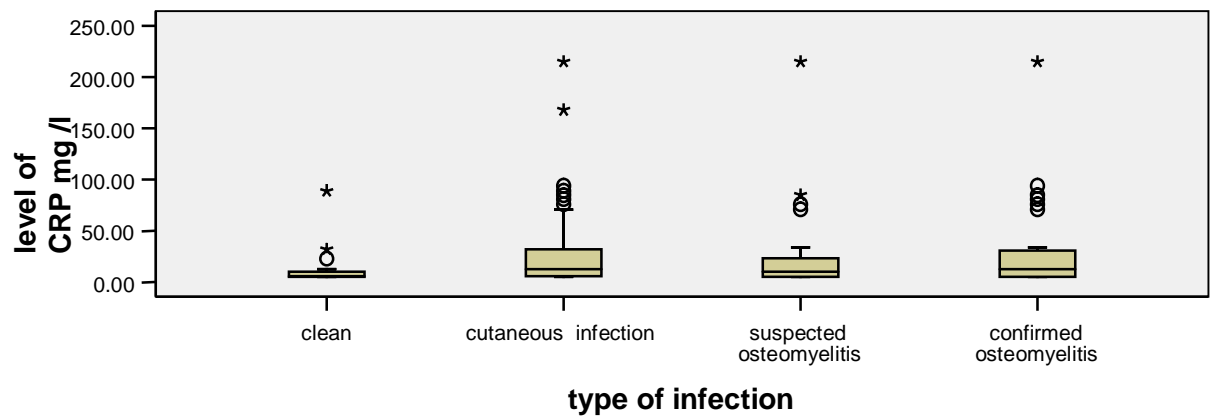


Figure 4.4.4 Comparison of Plasma Viscosity level in all wound conditions

Accepted normal 1.5 – 1.72 mpas

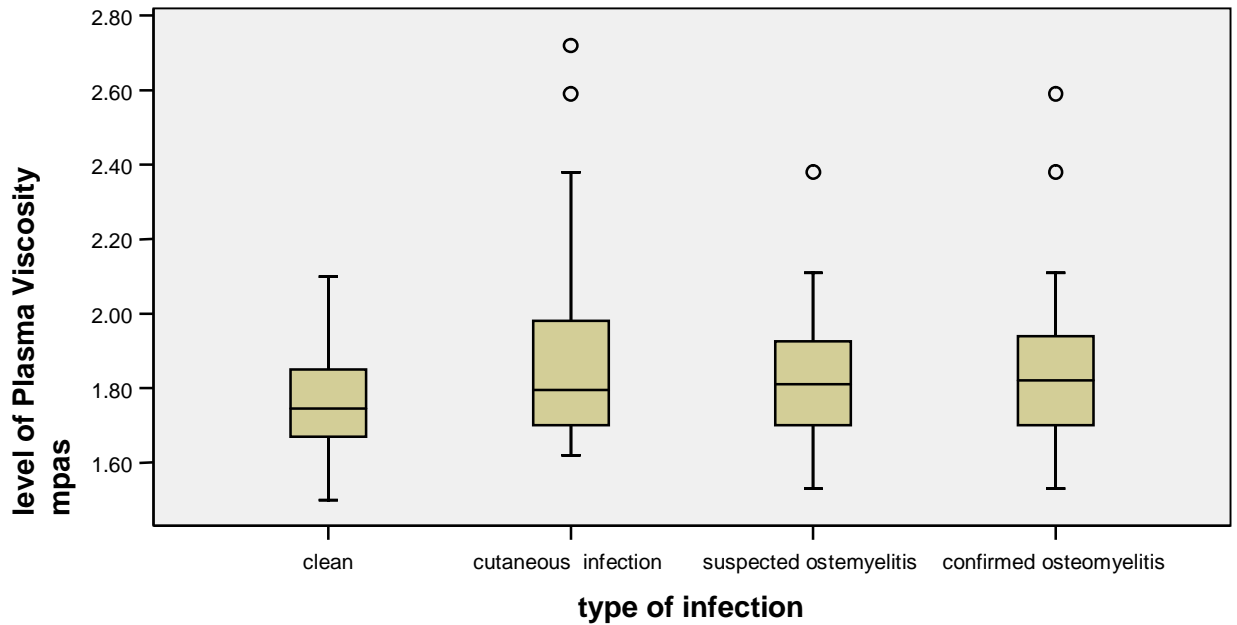
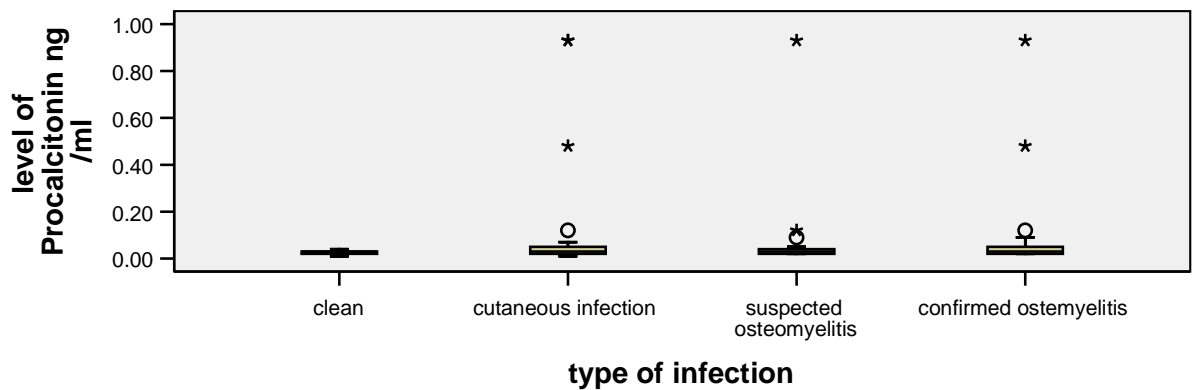


Figure 4.4.5 Comparison of Procalcitonin level in all wound conditions

Accepted normal in 'healthy' individuals <0.3ng/ml



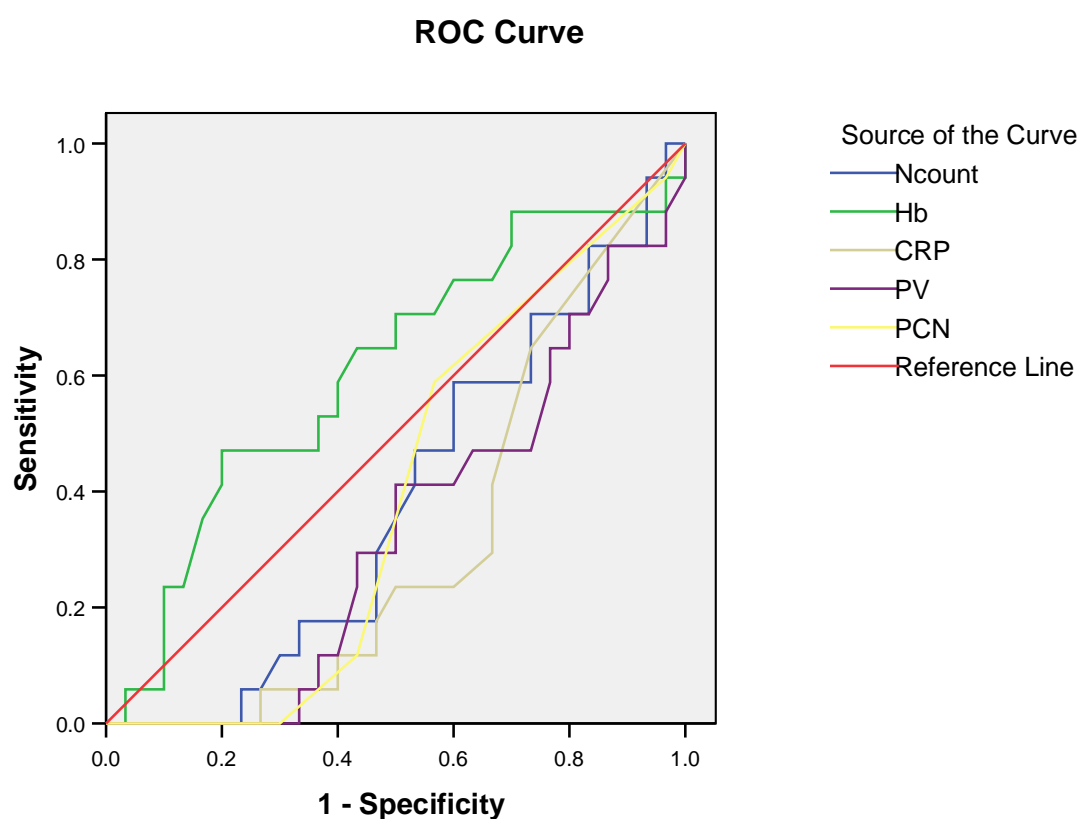
#### 4.5 Receiver Operator Characteristic Curves

Receiver operator characteristic curves are a plot of the sensitivity (detection rate) versus false positive rate (100 - specificity %) for selected cut off points. An ideal situation gives a result with the graph having a line up the y axis to near the top left corner and across parallel to the x axis – high detection rate with a low false positive rate. An ideal test would be both specific and sensitive.

Figure 4. 5.1 Receiver Operator Characteristic Curve – clean wounds

This is for each inflammatory blood marker in clean wounds

(With Haemoglobin excluding potential renal anaemia)



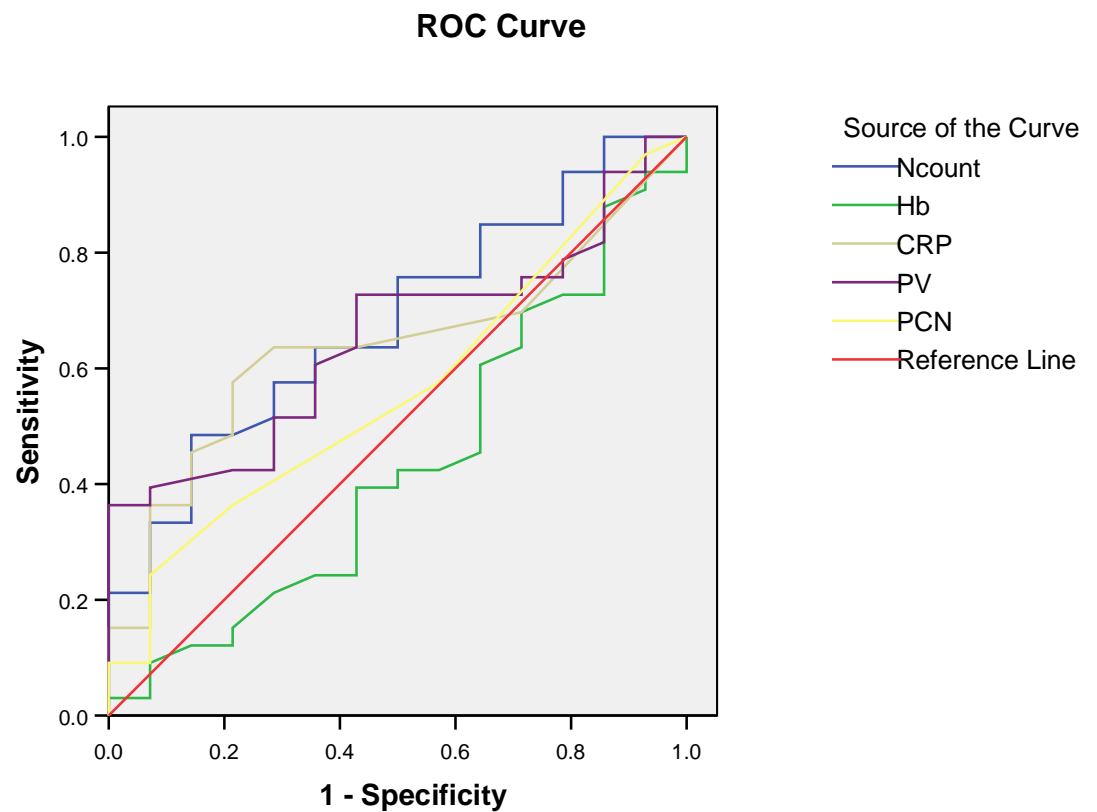
Diagonal segments are produced by ties.

**Area Under the Curve**

Test Result Variable(s)	Area
Neutrophil Count	.383
Haemoglobin	.610
C Reactive Protein	.319
Plasma Viscosity	.334
Procalcitonin	.393

**Figure 4.5.2 Receiver Operator Characteristic Curve – cutaneous infection in wounds**

This is for each inflammatory blood marker in cutaneously infected wounds  
(With Haemoglobin excluding potential renal anaemia)



Diagonal segments are produced by ties.

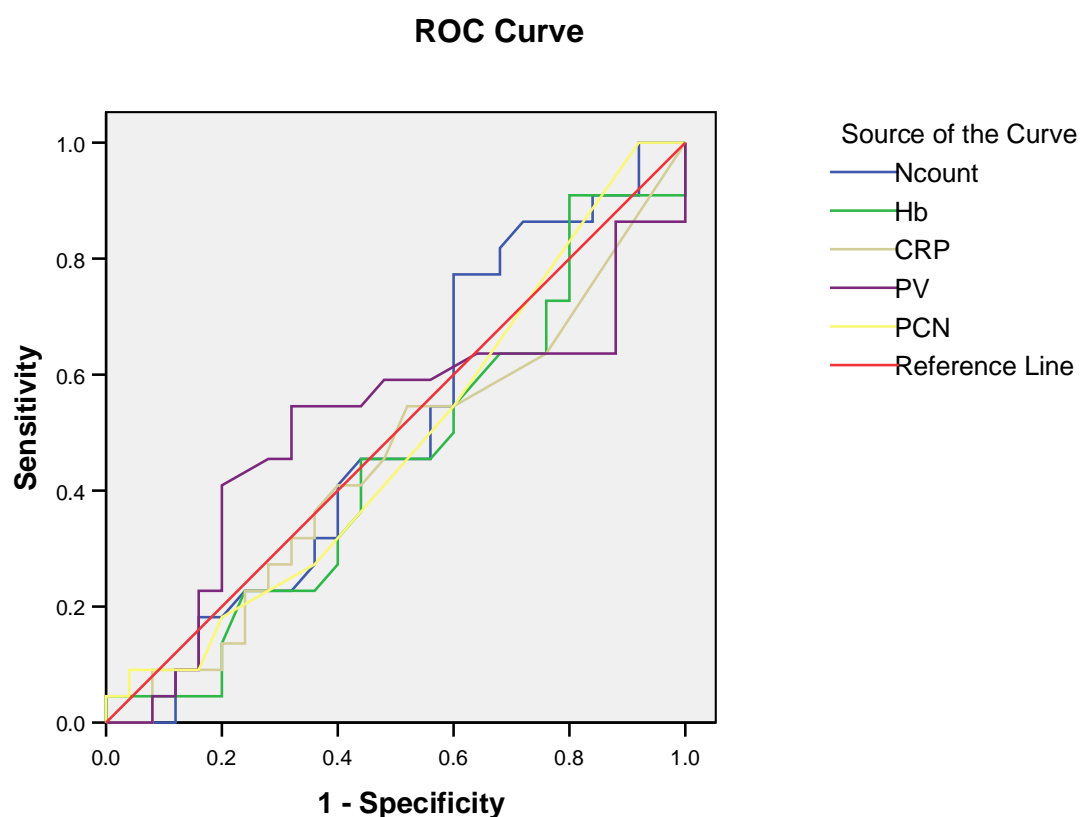
**Area Under the Curve**

Test Result Variable(s)	Area
Neutrophil Count	.681
Haemoglobin	.433
C Reactive Protein	.637
Plasma Viscosity	.654
Procalcitonin	.564



Figure 4.5.3 Receiver Operator Characteristic Curve – suspected osteomyelitis infected wounds

This is for each inflammatory blood marker in suspected osteomyelitis infected wounds (With Haemoglobin excluding potential renal anaemia)



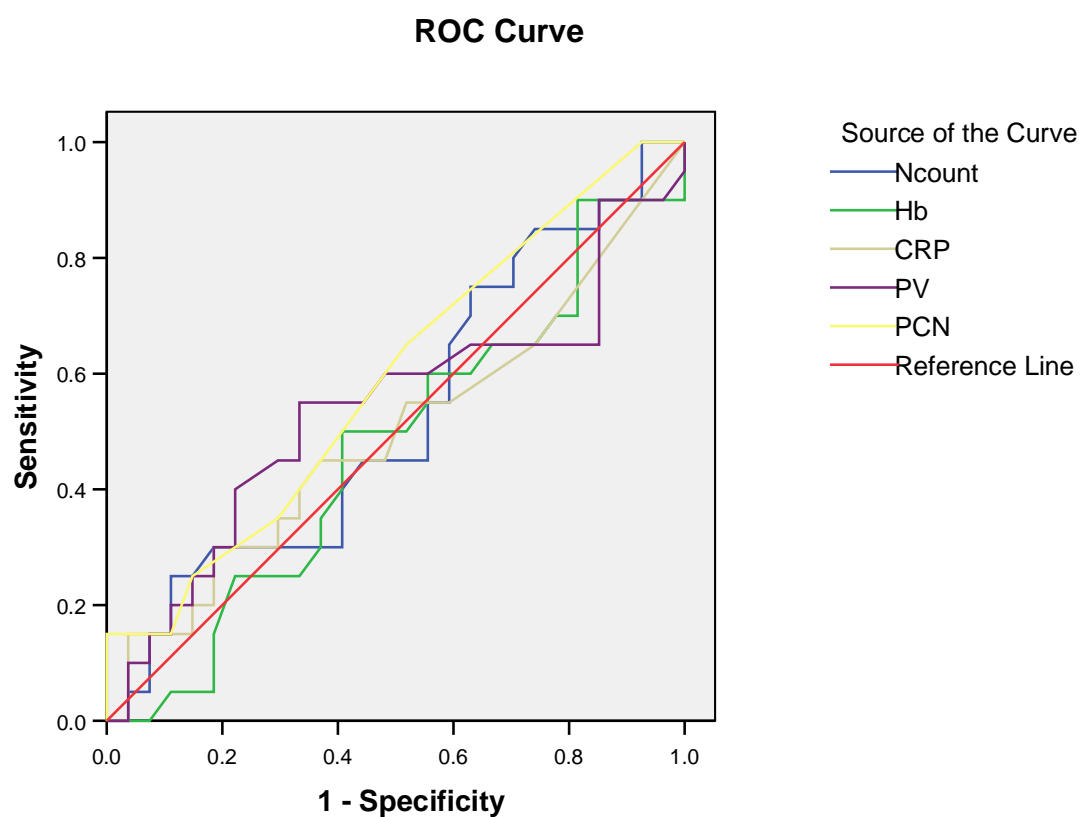
Diagonal segments are produced by ties.

**Area Under the Curve**

Test Result Variable(s)	Area
Neutrophil Count	.505
Haemoglobin	.450
C Reactive Protein	.453
Plasma Viscosity	.508
Procalcitonin	.480

Figure 4.5.4 Receiver Operator Characteristic Curve – confirmed osteomyelitis infected wounds

This is for each inflammatory blood marker in proven osteomyelitis infected wounds  
(With Haemoglobin excluding potential renal anaemia)



Diagonal segments are produced by ties.

**Area Under the Curve**

Test Result Variable(s)	Area
Neutrophil Count	.526
Haemoglobin	.472
C Reactive Protein	.498
Plasma Viscosity	.537
Procalcitonin	.590

#### 4.6 Logistic Regression analysis without recognising confounders

Regression is a method whereby a statistical method is used to predict an outcome variable (in this study the outcome variable is the wound being clean, having a cutaneous infection, having suspected or proven osteomyelitis) by the use of predictor variables (the inflammatory blood marker level). The level of significance used was  $p \leq 0.5$ . The full results of the regression analysis can be found in Appendix 11.

Table 4.8 Logistic Regression analysis without recognising potential confounders predicting all wound types

<b>Wound type</b>	<b>Marker that is significant</b>	<b>level of significance p =</b>	<b>Exp(B)</b>	<b>95% Confidence intervals</b>
<b>Clean</b>	Plasma Viscosity	0.05	77.02	0.95, 6245.65
<b>Cutaneous infection</b>	Neutrophil Count	0.05	0.70	0.49, 0.99
<b>Suspected osteomyelitis</b>	None	N/A		
<b>Confirmed osteomyelitis</b>	None	N/A		

#### 4.7 Logistic Regression analysis with recognised potential confounders

The same regression technique was used and the confounders of antibiotic use, Insulin use, Metformin use and the wound being predominantly neuropathic in origin are now presented. The patients attending the Diabetes Foot Clinic may not have been prescribed antibiotics by the primary care team that had referred them so the apparent anomaly of not using antibiotics in infections is thus explained. The level of significance of less than 0.05 was requested in the determination of the results and as such some levels are greater than the accepted norm of  $p < 0.05$ . The level is of sufficient for the inflammatory marker to be included in an equation to predict a wound condition.

Table 4.9 Potential confounders in regression analysis predicting clean wounds

<b>Confounder</b>	<b>marker that is significant</b>	<b>level of significance p =</b>	<b>Exp(B)</b>	<b>95% Confidence intervals</b>
<b>no antibiotics</b>	C Reactive Protein	0.07	1.16	0.99, 1.36
<b>Insulin treatment</b>	None	N/A		
<b>Metformin treatment</b>	None	N/A		
<b>Etiology predominantly neuropathic</b>	None	N/A		

Table 4.10 Potential confounders in regression analysis predicting wounds with cutaneous infection

<b>confounder</b>	<b>marker that is significant</b>	<b>level of significance p =</b>	<b>Exp(B)</b>	<b>95% Confidence intervals</b>
<b>no antibiotics</b>	Neutrophil Count	0.09	0.54	0.27, 1.11
<b>Insulin treatment</b>	Neutrophil Count	0.05	0.56	0.33, 0.96
<b>Metformin treatment</b>	None	N/A		
<b>Etiology predominantly neuropathic</b>	Neutrophil Count	0.05	0.65	0.42, 0.99

Table 4.11 Potential confounders in regression analysis predicting wounds with suspected osteomyelitis

<b>confounder</b>	<b>marker that is significant</b>	<b>level of significance p =</b>	<b>Exp(B)</b>	<b>95% Confidence intervals</b>
<b>no antibiotics</b>	Plasma Viscosity	0.06	< 0.01	0.00, 1.70
<b>Insulin treatment</b>	None	N/A		
<b>Metformin treatment</b>	None	N/A		
<b>Etiology predominantly neuropathic</b>	None	N/A		

Table 4.12 Potential confounders in regression analysis predicting wounds with confirmed osteomyelitis

<b>confounder</b>	<b>marker that is significant</b>	<b>level of significance p =</b>	<b>Exp(B)</b>	<b>95% Confidence intervals</b>
<b>no antibiotics</b>	Plasma Viscosity	0.09	< 0.01	0.00, 4.10
<b>Insulin treatment</b>	None	N/A		
<b>Metformin treatment</b>	None	N/A		
<b>Etiology predominantly neuropathic</b>	None	N/A		

Table 4.13 Comparison of C Reactive Protein in all individuals with type 2 diabetes to those with type 2 diabetes AND on Metformin and BMI >25 kg / m<sup>2</sup> for all wound types

	<b>Type 2 diabetes</b>	<b>Type 2 diabetes AND on Metformin AND BMI ≥ 25 kg / m<sup>2</sup></b>
Mean (±Standard deviation)	23.76 (±35.82)	31.85 (±57.28)
Median (Interquartile range)	10.00(4.99, 26.00)	10.00 (4.99, 26.50)

The high probability of the level of each of the inflammatory markers being strongly correlated to at least one other marker is shown in the tables below.

Table 4.14 Correlations between inflammatory markers in clean wounds

	<b>Neutrophil Count</b>	<b>Haemoglobin</b>	<b>C Reactive Protein</b>	<b>Plasma Viscosity</b>	<b>Procalcitonin</b>
<b>Neutrophil Count</b>	1	- 0.048	0.666**	0.423*	0.546*
<b>Haemoglobin</b>	- 0.048	1	0.139	- 0.166	0.242
<b>C Reactive Protein</b>	0.666**	0.139	1	0.520**	0.228
<b>Plasma Viscosity</b>	0.423*	- 0.166	0.520**	1	- 0.217
<b>Procalcitonin</b>	0.546*	0.242	0.228	- 0.217	1

Correlation is Pearson correlation

\*\* Correlation is significant at  $p \leq 0.01$  level (2 tailed)

\* Correlation is significant at  $p \leq 0.05$  level (2 tailed)

Table 4.15 Correlations between inflammatory markers in wounds with cutaneous infection

	<b>Neutrophil Count</b>	<b>Haemoglobin</b>	<b>C Reactive Protein</b>	<b>Plasma Viscosity</b>	<b>Procalcitonin</b>
<b>Neutrophil Count</b>	1	- 0.042	0.513	0.426**	0.044
<b>Haemoglobin</b>	- 0.042	1	- 0.187	- 0.225	- 0.060
<b>C Reactive Protein</b>	0.513	-0.187	1	0.470**	0.204
<b>Plasma Viscosity</b>	0.426**	- 0.225	0.470**	1	0.141
<b>Procalcitonin</b>	0.044	- 0.060	0.204	0.141	1

Correlation is Pearson correlation

\*\* Correlation is significant at  $p \leq 0.01$  level (2 tailed)

\* Correlation is significant at  $p \leq 0.05$  level (2 tailed)

Table 4.16 Correlations between inflammatory markers in suspected osteomyelitis

	<b>Neutrophil Count</b>	<b>Haemoglobin</b>	<b>C Reactive Protein</b>	<b>Plasma Viscosity</b>	<b>Procalcitonin</b>
<b>Neutrophil Count</b>	1	- 0.343	0.582**	0.177	- 0.201
<b>Haemoglobin</b>	- 0.343	1	- 0.211	- 0.458*	- 0.245
<b>C Reactive Protein</b>	0.582**	- 0.211	1	0.432*	0.165
<b>Plasma Viscosity</b>	0.177	- 0.458*	0.432*	1	- 0.051
<b>Procalcitonin</b>	- 0.201	- 0.245	0.165	-0.051	1

Correlation is Pearson correlation

\*\* Correlation is significant at  $p \leq 0.01$  level (2 tailed)

\* Correlation is significant at  $p \leq 0.05$  level (2 tailed)

Table 4.17 Correlations between inflammatory markers in confirmed osteomyelitis

	<b>Neutrophil Count</b>	<b>Haemoglobin</b>	<b>C Reactive Protein</b>	<b>Plasma Viscosity</b>	<b>Procalcitonin</b>
<b>Neutrophil Count</b>	1	- 0.193	0.629**	0.341	0.081
<b>Haemoglobin</b>	- 0.193	1	- 0.102	- 0.124	- 0.064
<b>C Reactive Protein</b>	0.629**	- 0.102	1	0.510**	0.355
<b>Plasma Viscosity</b>	0.341	- 0.124	0.510**	1	0.225
<b>Procalcitonin</b>	0.081	- 0.064	0.355	0.225	1

Correlation is Pearson correlation

\* Correlation is significant at  $p \leq 0.01$  level (2 tailed)

\*\* Correlation is significant at  $p \leq 0.05$  level (2 tailed)



## **Chapter 5 Discussion The use of inflammatory blood markers in the diagnosis of osteomyelitis**

This chapter will discuss the results found and consider them in relation to the previous studies that have been identified. However it is worth restating the fact that none of the papers found to date about the use of inflammatory markers have considered using them in an out - patient setting.

### **5.1 Sample characteristics**

The sample in this research shows characteristics broadly similar to that of Eneroth , Larson and Apelqvist (1999) in terms of mean age, this study 66 years compared to 70 years, being older than that of Armstrong *et al* (1996b) – 58.7 - 62.3 years (3 centres) and Leichter *et al* (1988) – 53.5 years. These other papers are describing patients who had been admitted as a consequence of their foot lesion.

In terms of duration of diabetes Armstrong *et al* (1996b) shows his three centres to have a mean range of duration from 14.7 years to 15.8 years, in this study the mean was 14.9 years, similar and that of Lavery *et al* (2006) at 13.9 years.

The ‘excess’ male patients is a well recognized occurrence in diabetic foot disease being noted by amongst others Reiber (1996), El-Shazley *et al* (1998), Wikblad, Wibell and Montin (1990) and Benotame *et al* (2000).

Leichter *et al* (1988) describe a proportion of some 70% having type 2 diabetes as opposed to 80% in this study. The increase in patients with diabetes has seen the largest increase in type 2 diabetes in latter years and this maybe shown in part by the

larger numbers here presented.

The use of Insulin in treatment in this study – 61% is not comparable to that of Eneroth *et al* (1999) reporting 70% in infections that were either deep soft tissue or a combination it did not include the clean wounds of this study but more like that of Leichter *et al* (1988) reporting 63.6%.

The results may have been affected by the nature of the presenting problem in a number of individuals making them atypical out patients. Although seen in an out – patient setting there was a total of two who were being treated with home intra – venous antibiotics and this historically has been an in – patient procedure, one had an infection that the team would to have preferred to have treated as an in – patient but he refused admission and an additional two were admitted not from clinic but within a couple of days for surgical debridement and further investigations as to determine if the infection was local and contained within the skin or had progressed to bone infection. This was most noticeable in the results for Procalcitonin where the mean (with standard deviation) and median for cutaneous infection including these to not including them was 0.05 ng / ml ( $\pm$  0.08 ng /ml), median, 0.03 ng /ml (accepted normal 0.03 ng /ml), in suspected osteomyelitis 0.04 ng /ml ( $\pm$ 0.03 ng /ml), median 0.03 ng / ml and in proven osteomyelitis 0.06 ng /ml ( $\pm$ 0.10 ng /ml) median 0.03 ng /ml. The data presented has still included them in most results presented however it could be argued they should be excluded as they are in Table 4.7 on page 86.

## 5.2 Descriptive statistics of Inflammatory blood markers

The following is a discussion about the results of the study overall and not for each of the wound types that follows after. Table 4.7 on page 85 summarizes the range of the inflammatory blood markers results within this study with an accepted normal for comparison.

### 5.2.1 Neutrophil Count

The range and mean of the Neutrophil Count appears to be in line with the accepted normal of the pathology laboratory. This is with a group of individuals where infection is suspected in 63% so the level may have been expected to be high. The mean is seen to be towards the 'high end of normal'.

### 5.2.2 Haemoglobin

The Haemoglobin levels show the range to be generally at the 'lower end of normal' even when the potential for renal anaemia is excluded. The effect of chronic disease causing anaemia has been discussed previously. Chronic infection can add to this too and there was no effort made within this study to determine how long the wounds had been present. The level is approaching that considered to be indicative of anaemia of 11 g /dl by the World Health Organization.

### 5.2.3 C Reactive Protein

This study was limited by the pathology laboratory not being able to analyze C Reactive Protein below 5 mg / l for the biggest part of this study. This has limited the results in that many were reported as < 5 mg / l and considered to be 'normal' and the results are difficult to interpret from this. It also is a marker that, as can be seen, has a very large range. The mean is above the considered normal and this would be expected in wounds of any description.

### 5.2.4 Plasma Viscosity

The range of readings shows the mean to be just above that to be considered normal. This is discussed in more detail in the section about confounders in regression as it is recognized that the haematological determinants of blood flow resistance do have some association with endocrine and vascular disorders (MacRury *et al* 1990 and Coppola *et al* 1997), namely neuropathy and Insulin in type 2 diabetes.

#### 5.2.5 Procalcitonin

To date little is known about the effect of diabetic foot ulceration on levels of Procalcitonin as it has been used more commonly in Systemic Inflammatory Response Syndrome. There were five individuals in this study that although they were being treated in an out patients setting in – patient care may have been more usual and this may have had a profound effect on the results. Removing these individuals the remainders appear to be contrary to the work reported by both Uzun *et al* (2007) and Jeandrot *et al* (2008). The median (used as the central point as in the two studies mentioned above) does not appear to rise in the series presented here from clean wounds to those with proven osteomyelitis. (The atypical use of an out-patients setting is discussed in more detail in the section about sample characteristics.)

#### 5.3 Inflammatory blood markers in clean wounds

The mean Neutrophil Count is within the ‘normal’ range expected when no infection is present. The Haemoglobin is within the ‘normal’ range. C Reactive Protein shows some rise compared to ‘normal’ and this maybe due to the disease process of diabetes being an inflammatory process which is discussed later. Plasma Viscosity is close to ‘normal’ when looking at the mean, Coppola *et al* (1997) has shown this high end of ‘normal’ to be present in type 2 diabetes and with Insulin use. MacRury *et al* (1990) had shown the rise of Plasma Viscosity to be associated with neuropathy in diabetes. Individuals with foot wounds associated with diabetes would be expected to show some degree of neuropathy. The mean Procalcitonin level is as would be expected low.

#### 5.4 Inflammatory blood markers in cutaneous infection

The expected pattern when infection occurs would be for Haemoglobin to reduce and

all other markers to rise.

The Neutrophil Count is seen to have risen compared to clean wounds but is still within the 'normal' range. Most diabetic foot ulcers, when showing signs of infection, are infected by bacteria. As neutrophils are the cell responsible for bacterial killing it would be expected that a rise above 'normal' would occur. This maybe from the recognized defects in leucocyte chemotactic factors that have been observed when a poor inflammatory response occurs (Bagdale, Root and Bulger 1974, Tan *et al* 1975 and Molenaar *et al* 1976). The poor inflammatory response is a complication associated with chronic hyperglycaemia and includes the prime problems of diabetic foot disease, namely neuropathy and ischaemia.

C Reactive Protein shows a rise that is consistent with infection causing an acute phase reaction to occur when comparing both mean and median to 'normal', normal <5 mg/l, mean 28.16 mg /l, median 13.00 mg /l.

Plasma Viscosity rises a little more than in clean wounds, the change is small.

Procalcitonin rises but is still at a level that is considered to be 'normal'.

As has been discussed in the introduction questions have been raised about the response of people with diabetes to develop these markers (Armstrong *et al* 1996 and Leichter *et al* 1998, Oncul *et al* 2006). It has been recognized that the cardinal signs of infection can be both mimicked and obscured by the diabetes associated complications of ischaemia or neuropathy (Cavanagh *et al* 2005). The changes in the microvessels affect both delivery of the cells required and the inflammatory response (Sannomyia, Pereira and Garcia-Leme 1990).

### 5.5 Inflammatory blood markers in suspected osteomyelitis

None of the markers show any significant change between cutaneous infection and suspected osteomyelitis. From this study it is unclear if the use of antibiotic therapy has reduced the inflammatory response and as such the markers do not show any change.

### 5.6 Inflammatory blood markers in proven osteomyelitis

Again none of the markers show any significant change between cutaneous infection and suspected osteomyelitis. From this study it is unclear if the use of antibiotic therapy has reduced the inflammatory response and as such the markers do not show any change.

The lack of change in levels of the markers maybe affected by the disease process of diabetes as discussed above and in the introduction or due to the use antibiotics

### 5.7 Receiver Operator Characteristic curve in clean wounds

None of the inflammatory markers show any sign of being of potential use with clean wounds. The lack of both sensitivity and specificity is seen by the lines being close to the reference line. Haemoglobin shows a low sensitivity at the 'highest' point of about 40% with a specificity of 80%. The other markers are both less sensitive and specific.

### 5.8 Receiver Operator Characteristic curve in cutaneous infection

The influence of infection on Haemoglobin appears to be evident in this Receiver Operator Characteristic (ROC) curve in this in comparison to that for clean wounds

in that the Haemoglobin in that it is becoming less sensitive. The other markers are nearer the area of the curve where a useful marker would be – the top right being both sensitive and specific. The Area Under the Curve statistic is better when the value is nearer 1. Plasma Viscosity shows the greatest Area Under the Curve at 0.654.

Neutrophil Count, C Reactive Protein and Plasma Viscosity all show a sensitivity of approximately 60% with a specificity of 30% at best. These may be useful when combined with clinical findings.

### 5.9 Receiver Operator Characteristic curve in suspected osteomyelitis

As in the clean wounds no marker shows any sign of being of potential benefit with Plasma Viscosity showing the best result at 55% sensitivity and 30% specificity or 60% sensitive and 90% specific. This may be useful when combined with clinical suspicion. However the Neutrophil Count provides a greater Area Under the Curve of 0.505 with the sensitivity of 85% and specificity of 70%.

### 5.10 Receiver Operator Characteristic curve in proven osteomyelitis

The markers seem to show an equal non specific and non sensitivity to confirmed osteomyelitis.

None of the ROC curves show any outstanding benefit of using a marker in the diagnosis of a cutaneous or bone infection in this series.

### 5.11 Regression models with no confounders in clean wounds

The regression model shows Plasma Viscosity to be predictive of the condition

unlike the Receiver Operator Characteristic curve. Plasma Viscosity in this model has a statistical significance of  $p = 0.053$ .

### 5.12 Regression models with no confounders in cutaneous infection

Neutrophil Count has a statistical significance of  $p = 0.08$  and is predictive of the condition.

### 5.13 Regression models with no confounders in suspected osteomyelitis

No marker is of clear use with levels of significance far above the accepted  $p < 0.05$ .

### 5.14 Regression models with no confounders in proven osteomyelitis

No marker is of clear use with levels of significance far above the accepted  $p < 0.05$ .

### 5.15 Regression models with confounders in clean wounds

#### 5.15.1 No antibiotic use

C Reactive Protein is marginally statistically significant, ( $p = 0.07$ ) and is recognized as being of sufficient value to add to any equation to determine a clean wound. This would follow the argument of Upchurch *et al* 1997) that diabetes *and* (my emphasis) having a foot wound raises the C Reactive Protein level.



#### 5.15.2 Use of Insulin

Neutrophil Count is significant and recognized as being of sufficient value to add to any equation to determine a clean wound ( $p = 0.05$ ).

Plasma Viscosity is no longer significant ( $p = 0.29$  Insulin use vs.  $p = 0.05$  no confounders) and is not recognized as being of sufficient value to add to any equation to determine a clean wound as suggested by Coppola *et al* (1997).

#### 5.15.3 Use of Metformin

No marker is significant.

#### 5.15.4 Predominantly neuropathic wounds

No marker is significant Plasma Viscosity is less significant ( $p = 0.07$  neuropathic vs.  $p=0.05$  no confounder) in agreement with the suggestion by MacRury *et al* (1990).

C Reactive Protein is still no longer significant ( $p = 0.05$  neuropathic vs.  $p = 0.13$  no confounder). This would follow the argument of Upchurch *et al* 1997) that diabetes *and* (my emphasis) having a foot wound raises the C Reactive Protein level. If it can be argued that ischaemia means C Reactive Protein rises then a predominantly neuropathic wound with a lesser influence by ischaemia then C Reactive Protein should reduce this result is counter to the argument of Yu *et al* (2004).

## 5.16 Regression models with confounders in cutaneous infection

### 5.16.1 No antibiotic use

Neutrophil Count remains significant, within the equation but not statistically, ( $p = 0.09$  no antibiotic use *vs.*  $p = 0.05$  no confounder) and is predictive. Pittet *et al* (1999) suggested Neutrophil Count was not able to predict outcome of foot lesions.

C Reactive Protein remains non significant ( $p=0.24$  no antibiotic *vs.*  $p = 0.19$  no confounder).

Plasma Viscosity remains non significant ( $p = 0.19$  no antibiotic *vs.*  $p = 0.20$  no confounder).

### 5.16.2 Use of Insulin

Neutrophil Count remains significant ( $p = 0.04$  Insulin use *vs.*  $p = 0.05$  no confounder) and is still considered to be in an equation to determine cutaneous infection. Again this is counter to the work of Pittet *et al* (1999) suggesting Neutrophil Count was not able to predict outcome of foot lesions.

C Reactive Protein is not of a level to be significant in the equation ( $p = 0.04$  Insulin use *vs.*  $p = 0.19$  no confounder).

Plasma Viscosity is not of a level to be significant ( $p = 0.09$  Insulin use *vs.*  $p = 0.20$

no confounder) but has become of marginal significance as suggested by Coppola *et al* (1997).

#### 5.16.3 Use of Metformin

No marker is predictive of the condition.

Neutrophil Count is no longer significant ( $p = 0.42$  Metformin use vs.  $p = 0.05$  no confounder). This follows the work of Pittet *et al* (1999) suggesting Neutrophil Count was not able to predict outcome of foot lesions.

C Reactive Protein is not significant ( $p = 0.20$  Metformin use vs.  $p = 0.19$  no confounder). It is known that 24 individuals in this study were 'overweight' and had 'type 2 diabetes' together, this follows the suggestion by Carter *et al* (2005) that Metformin reduces C Reactive Protein. The Carter *et al (ibid)* study was however on individuals with type 2 and 'overweight' and on Metformin, in this study of osteomyelitis only 14 individuals had all three characteristics making any inference lacking in power.

Plasma Viscosity is not significant ( $p = 0.32$  Metformin use vs.  $p = 0.20$  no confounder).

#### 5.16.4 Predominantly neuropathic wounds

Neutrophil Count keeps the same significance ( $p = 0.05$  neuropathic vs.  $p = 0.05$  no confounder) and remains in the equation to predict cutaneous infection. Again this is counter to the work of Pittet *et al* (1999) suggesting Neutrophil Count was not able to predict outcome of foot lesions.

C Reactive Protein remains of non significance ( $p = 0.12$  no confounder vs.  $p = 0.16$  neuropathic). It is known a total of 24 individuals in this study were 'overweight' and had 'type 2 diabetes' together, and 14 were all three of type 2, 'overweight' and took Metformin, this follows the suggestion by Carter *et al* (2005) that Metformin reduces C Reactive Protein. As in clean wounds if it can be argued that ischaemia means C Reactive Protein rises then a predominantly neuropathic wound with a lesser influence by ischaemia then C Reactive Protein should reduce this result is again counter to the argument of Yu *et al* (2004).

Plasma Viscosity is not significant ( $p = 0.02$  vs.  $p = 0.14$ ) as suggested by MacRury *et al* (1990).

### 5.17 Regression models with confounders in suspected osteomyelitis

#### 5.17.1 No antibiotic use

Neutrophil Count is not significant ( $p = 0.65$  no antibiotic vs.  $p = 0.76$  no confounder). Again this follows the work of Pittet *et al* (1999) suggesting Neutrophil Count was not able to predict outcome of foot lesions.

Plasma Viscosity is significant with being considered to be in an equation to determine suspected osteomyelitis ( $p = 0.06$  no antibiotic vs.  $p = 0.63$  no confounder).

#### 5.17.2 Use of Insulin

Plasma Viscosity is not significant with not being considered to be in an equation to determine suspected osteomyelitis ( $p = 0.83$  Insulin use vs.  $p = 0.63$  no confounder),

as suggested by Coppola *et al* (1990).

#### 5.17.3 Use of Metformin

Plasma Viscosity is not significant with no longer being considered to be in an equation to determine suspected osteomyelitis ( $p = 0.71$  Metformin use *vs.*  $p = 0.63$  no confounder).

#### 5.17.4 Predominantly neuropathic wounds

Plasma Viscosity is not significant with no longer being considered to be in an equation to determine suspected osteomyelitis ( $p = 0.94$  neuropathic *vs.*  $p = 0.63$  no confounder), counter to the suggestion by MacRury *et al* (1997).

### 5.18 Regression models with confounders in proven osteomyelitis

#### 5.18.1 No antibiotic use

Neutrophil Count is not significant ( $p = 0.81$  no antibiotic *vs.*  $p = 0.72$  no confounder). Again this follows the work of Pittet *et al* (1999) suggesting Neutrophil Count was not able to predict outcome of foot lesions.

Plasma Viscosity becomes significant and can predict the condition ( $p = 0.09$  no antibiotic *vs.*  $p = 0.65$  no confounder).

#### 5.18.2 Use of Insulin

All markers remain not significant.

#### 5.18.3 Use of Metformin

All markers remain not significant. It is not known only 14 in this study were 'overweight', had 'type 2 diabetes' and took Metformin this may follow the suggestion by Carter *et al* (2005) that Metformin reduces C Reactive Protein,  $p = 0.77$  no confounder *vs.*  $p = 0.61$  Metformin use.

#### 5.18.4 Predominantly neuropathic wounds

All markers remain not significant.

This is against the work of Katsaros *et al* (2008), Jeandrot *et al* (2008) and Fleischer *et al* (2009) who all claim an increase in markers particularly C Reactive Protein in osteomyelitis ( $p = 0.89$  neuropathic *vs.*  $p = 0.77$  no confounders).

### 5.19 Comparative results to other studies for Neutrophil Count

Neutrophil Count is not commonly used in previous studies. The study by Al-Gwaiz and Babay (2007) was looking at patients with a positive blood culture for bacterial infection as and such is not directly comparable to this study. The work they produced suggests that a 'severe' infection, with no definition, can be recognised by an absolute Neutrophil Count to be  $>8.0 \times 10^9 / l$ , this was not achieved in comparing

the mean values in any of the groups in this research. The maximum in both the suspected and proven osteomyelitis group did however reach this level.

Tan *et al* (1975) suggested the inefficient neutrophil capacity in diabetes maybe associated with the increased susceptibility to infection. This paper was however considering the impairment of intracellular killing by phagocytosis and did not examine if this was related to low neutrophil numbers as well as poor activity.

The work by Pittet *et al* (1999) was in diabetic foot infections and on patients that had been admitted as a consequence of their foot problem so is not directly comparable to this work. Pittet *et al* (1999) found a Neutrophil Count failed to predict the outcome in 105 patients including 55% with suspected deep tissue and osteomyelitis combined. In this thesis Neutrophil Count was the most useful marker in prediction of cutaneous infection across all confounding groups (and no confounders) with the exception of Metformin use.

The apparent lack of change in the mean and standard deviation of the four groups within this research may agree with the work by Armstrong *et al* (1996b) studying 338 consecutive admissions for a primary diagnosis of infected foot ulceration associated with diabetes showed 56% to have a total white cell count within their 'normal' limit.

The work of Uzun *et al* (2007) shows a total white blood cell count was found to be higher comparing the mean count in a control group to those with an infection but not comparing a non infected diabetic foot wound to an infected one. This latter paper suggests that a rise in level does occur in infections.

## 5.20 Comparative results to other studies for Haemoglobin

The recognised potential effect of renal disease on the capacity to produce erythropoietin (Thomas and Rampersad 2004) has meant that for the results this has

been removed and the discussion is related to the remaining 83 individuals in this research.

The effect of diabetes alone without compromised renal function anaemia seems to give a conflicting prevalence of anaemia from 10%; Stevens *et al* (2003), 23%; Thomas *et al* (2003) to 50% by Bosman *et al* (2001).

A foot infection in diabetes reduces Haemoglobin statistically compared to patients with diabetes and no foot ulcer and patients (as they were hospitalized but unclear why) without diabetes in a study by Upchurch *et al* (1997), again this is a study performed on patients admitted to hospital as a result of their foot problem.

This study reported here about inflammatory markers shows a gradual decline in mean Haemoglobin from clean wounds through the spectrum to confirmed osteomyelitis. It is not possible to tell if this is the anaemia of chronic disease with no data collected to compare with an analysis of the duration of disease specifically with the different wound conditions.

### 5.21 Comparative results to other studies for C Reactive Protein

C Reactive Protein is commonly used in studies and as a result this section will be discussing other studies found to offer additional materials to this report are given in date order.

Upchurch *et al* (1997) showed the rise of C Reactive Protein associated with both having diabetes and having a foot ulcer associated with diabetes. The assay method was not comparable to that used in this study with reports of diabetes and infected foot ulcer of a level of 5.6 mg /ml, diabetes alone 0.78 mg /ml, no diabetes



0.71 mg /ml. This appears to be the start of considering the acute phase reaction as being a quantifiable entity that may assist in recognition of the severity of foot disease.

Yu *et al* (2004) used C Reactive Protein as a predictor of peripheral vascular disease in diabetes. The study was cross sectional and concluded that a higher C Reactive Protein level (one at 0.282 mg / dl) was indicative of peripheral vascular disease in type 2 diabetes. Peripheral vascular disease is large component of diabetic foot disease and it is not clear how these relate to each other. This study about inflammatory markers does show a rise in level of C Reactive Protein (both mean and median) in clean wounds compared to 'normal' (<5g /l) mean 6mg/ l, median 11 mg / l and across the range from clean to confirmed osteomyelitis mean 13g / l, median 29.33g/ l but has no comparable work for 'normal' level in diabetes without active foot disease. Nesto (2004) suggests that the rise in C Reactive Protein in diabetes is a result of the disease having chronic sub clinical inflammation as a major component. Atherosclerosis also placed within this group of diseases.

Katsaros *et al* (2008) using MRI as the gold standard diagnostic test for osteomyelitis in a study of 64 patients found C Reactive Protein to be significantly raised in osteomyelitis. The pre test probability in this study is not discussed as in many of the other studies it is based on patients admitted to hospital as a direct consequence of the foot infection.

Jeandrot *et al* (2008) with the antibiotic naive patients with foot ulcers in comparison to ulcer free patients found C Reactive Protein to be of use in distinguishing infected from non-infected ulcers. (The study claims to have matched ulcer patients to controls in terms of peripheral and / or cardiovascular disease and neuropathy however there is no definition of both peripheral or cardiovascular disease and neuropathy.) The rise found in the Jeandrot *et al* (*ibid*) study level was continuous from ulcer free to severe, limb threatening, infection (grade 4 of the Infectious Diseases Society of America guidelines) but only significantly between cutaneous

infection and control and cutaneous infection and clean. This inflammatory blood marker study presented is difficult to compare to that of Jeandrot *et al (ibid)* as the use of antibiotics was not easily separated but has been discussed in the regression section with confounders.

The rise in C Reactive Protein in osteomyelitis is also noted by Fleischer *et al* (2009), again in hospitalized patients. Fleischer *et al (ibid)* advocate that a C Reactive Protein, in conjunction with assessment of ulcer depth, is a sensitive strategy for determining early osteomyelitis. The nature of the study was to detect early disease as determined and confirmed by histological examination of bone samples. This again is a difference that some of the patients in this thesis report may have had chronic osteomyelitis by the histological definition used by Fleischer *et al (ibid)*.

## 5.22 Comparative results to other studies for Plasma Viscosity

MacRury *et al* (1990) performed a study to examine the role of blood viscosity in peripheral neuropathy associated with diabetes. Diabetes had a statistically significant effect raising the mean level of Plasma Viscosity compared to controls. The rise was for all people with diabetes, the rise associated with diabetes and neuropathy as a separate entity was not statistically significant. In this study reported in this thesis over the entire group the mean was above the ‘normal’ limit and above ‘normal’ in all wound conditions i.e. clean, cutaneous infection, suspected and confirmed osteomyelitis. There was however little rise between the different infective states. Neuropathy was defined in a clinically meaningful way that meant it was applicable to the definition of neuropathy used here.

The significance reported in the regression studies for Plasma Viscosity is seen to reduce when the infection changes in the series presented in this work from clean to

cutaneous infection to suspected osteomyelitis to confirmed osteomyelitis. It is not clear what link there is between neuropathy and ‘severity’ of infection.

Coppola *et al* (1997) showed abnormalities in blood viscosity in type 2 diabetes and the use of Insulin but it is unclear as to cause and effect. The effect was significant in those patients with diabetes and evidence of vascular disease as opposed to ‘healthy’ i.e. non diabetic subjects. In this thesis the majority of individuals had evidence of vascular disease and it was significant in the majority of the foot ulcers few were predominantly neuropathic in origin. The definition of cardiovascular disease was far more complex in the Coppola study but did include history which although not defined may include the use of medication as used in the definitions in this thesis.

The significance of Insulin as a confounder on the marker of Plasma Viscosity is not clear from the regression studies, it is certainly not linear.

Plasma Viscosity in this reported thesis was predictive in the equation to suggest both suspected and confirmed osteomyelitis and showed the greatest Area Under the Curve in the ROC curve for suspected osteomyelitis and was the second highest Area Under the Curve in the ROC curve for confirmed osteomyelitis.

### 5.23 Comparative results to other studies for Procalcitonin

The two studies to date examining the use of Procalcitonin in diabetic foot ulceration have been in patients admitted to hospital. That by Uzun *et al* (2007) used a prospective method whereby the patients were determined to have, or not have, an infection or not using the criteria suggested by Lipsky *et al* (2004) for the Infectious Diseases Society of America guidelines (validated by Lavery *et al* 2007) similar numbers were noted to be taking antibiotics on admission when the blood samples

were taken. The group with an infected ulcer had a Procalcitonin mean level of 0.18ng / ml higher than that suggested by this study. The assay however does not appear to be as sensitive as that used here as the lower detection limit was 0.06 ng /ml as opposed to 0.01ng / ml. The patients in this study were all hospitalized – a difference to this here reported study where the majority were out patients and would have been treated that way, five it could be argued were atypical out patients (see section 5.1).

The paper by Jeandrot *et al* (2008) also used the Infectious Diseases Society of America guidelines as a defining infection protocol and again a different assay where the functional sensitivity was reported as 0.04 mg /ml however they report levels from 0.0 to 0.84 ng / ml. The 0.0 ng / ml is in a control group. A statistically significant rise is noted in the control and grade 1 ulcers and between grade 1 and grade 2. Grade 1 is no infection; grade 2 is skin and sub cutaneous tissue similar to the cutaneous infection group in this research. The patients were different from this research in that they were antibiotic naive for 6 months prior to the research and also in the fact they were admitted to hospital for the foot lesion. The difference between the clean and cutaneous infection groups in this research compared to the Jeandrot *et al* work maybe the unknown and quantifiable nature of antibiotic use (see later).

## 5.24 Comparative results for other confounders

### 5.24.1 Metformin use

Chu *et al* (2002) have shown in a study about cardiovascular risk factors that a statistically significant reduction in C Reactive Protein can occur after taking Metformin for a period as short as 4 months. The number of individuals taking Metformin in this study about osteomyelitis and inflammatory blood markers did note the taking of the drug but not the duration of treatment. The reduction in C

Reactive Protein was the only affect of Metformin produced that is of relevance to this osteomyelitis study and regrettably the data that would be required to determine if the individuals taking the drug have a lower level compared to those not taking it are not available. The Chu *et al (ibid)* study also used Metformin to achieve an agreed fasting blood glucose level that again was not noted within this study on osteomyelitis.

Carter *et al* (2005) suggested that Metformin dampened down chronic inflammation and as such C Reactive Protein synthesis or secretion without effect on glycaemic control in 'overweight' patients. This was not seen in this research on osteomyelitis comparing the C Reactive Protein levels as measured by mean (and standard deviation) and median (and interquartile range) of the two groups.

The nature of foot disease and chronic nature suggests that these studies may show some confounding of the results but in an unquantifiable way due to the unknown nature of Metformin use.

#### 5.24.2 Antibiotic use

The use of antibiotics to treat any infections would be expected to alter the inflammatory reaction. The use of antibiotics in all other papers has not been described adequately for the analysis of my results to be divided into groups for comparison with no antibiotic therapy, oral antibiotics and intra – venous antibiotics. The change of practice whereby we are now able to send patients home with intra - venous antibiotics may make the out patient setting described here more like the in - patient setting of the other papers.

When antibiotics were not being prescribed in cutaneous infection C Reactive Protein was the most significant inflammatory marker with  $p = 0.074$ , this had the effect of changing the significance by minus 0.005 when removed from the equation but did not affect the overall result to make any other marker of more benefit.

Neutrophil Count was the most significant in suspected osteomyelitis when antibiotics were not prescribed ( $p = 0.042$ ) followed by Plasma Viscosity ( $p = 0.60$ ). Neutrophil Count remained the most significant in proven osteomyelitis ( $p=0.095$ ) with a change when removed of ( $p = 0.030$ ).

No one single, or indeed any combination of, markers has shown to be of benefit in the detection of osteomyelitis associated with foot ulcers in diabetes. The multiple confounding factors do not help.

### 5.25 Correlation effect of inflammatory markers with no confounders

#### 5.25.1 Clean wounds

There is a highly significant statistical correlation in two areas; between Neutrophil Count and C Reactive Protein ( $p \leq 0.001$ ) and between Plasma Viscosity and C Reactive Protein ( $p \leq 0.001$ ). By examining the correlation coefficient it can be seen that the most relevant clinically is the relationship between Neutrophil Count and C Reactive Protein with 44% of each being due to the relationship, Plasma Viscosity and C Reactive Protein still has a noticeable inter relationship with an  $r^2$  value of 27%.

Neutrophil Count is also statistically significant relationship with both Plasma Viscosity and Procalcitonin (both  $\leq 0.005$ ). Clinically (by the  $r^2$ ) relationship this is only evident in the Plasma Viscosity correlation.

All correlations in this wound type were positive – as one marker increased so did the other.

This interrelationship of the markers is seen by the clustering appearance of these within the Receiver Operator Curve. No variable was considered in regression.

#### 5.25.2 Cutaneous infection

Neutrophil Count was found, again, to be statistically significantly correlated with C Reactive Protein and Plasma Viscosity. The clinical association remained at the same level (18%) between Neutrophil Count and Plasma Viscosity as in clean wounds. The clinical relationship with C Reactive Protein was higher at  $r^2 = 26\%$ .

C Reactive Protein and Plasma Viscosity continue to have a statistically significant interrelationship ( $p \leq 0.01$ ) with a clinical significance of 22%.

All statistically significant correlations in this wound type were positive – as one marker increased so did the other. There is a negative correlation between Neutrophil Count, C Reactive Protein, Plasma Viscosity, Procalcitonin and Haemoglobin levels.

This interrelationship of the markers is seen by the clustering appearance of these within the Receiver Operator Curve and separation of the Haemoglobin graph. No variable was considered in regression.

#### 5.25.3 Suspected osteomyelitis

Neutrophil Count and C Reactive Protein remain highly statistically significantly correlated ( $p \leq 0.001$ ) and clinically too with a clinical significance of 34%.

The significant statistical correlation continues to exist between Plasma Viscosity and C Reactive Protein ( $p \leq 0.005$ ) which is clinically positive – an  $r^2$  value of 26% and between Haemoglobin and Plasma Viscosity ( $p \leq 0.005$ ) but clinically this is seen to be a negative correlation (92%). Plasma Viscosity was the variable to be significant in the regression model.

The other markers (Neutrophil Count, C Reactive Protein and Procalcitonin) also show this negative correlation but not to a statistically significant level. The divergence of Haemoglobin is seen in the Receiver Operator Curve.

#### 5.25.4 Confirmed osteomyelitis

Neutrophil Count and C Reactive Protein remain highly statistically significantly correlated ( $p \leq 0.001$ ) and clinically too with a higher clinical significance of 39%.

C Reactive Protein and Plasma Viscosity continue to have a statistically significant interrelationship ( $p \leq 0.01$ ) with a clinical significance of 26%.

The other markers (Neutrophil Count, C Reactive Protein and Procalcitonin) also show this negative correlation but not to a statistically significant level. The divergence of Haemoglobin is seen in the Receiver Operator Curve. No variable was considered in regression.

### 5.26 Correlation effect of inflammatory markers with confounders

#### 5.26.1 Clean wounds

No marker was evident as being of greater significance within clean wounds and this maybe due to the noted statistically significant correlations between all the markers, except Haemoglobin, as noted in the regression model.

#### 5.26.2 Cutaneous infection

C Reactive Protein was the variable in the regression that was considered to be most likely to in the model when antibiotics were not used, it can be seen to be highly statistically and clinically correlated with both the Neutrophil Count and Plasma Viscosity in the correlation table.



The use of Insulin is important in the regression model for clean wounds and is seen as above to be clinically associated with C Reactive Protein and Plasma Viscosity.

#### 5.26.3 Suspected osteomyelitis

No marker was evident as being of greater significance within suspected osteomyelitis; C Reactive Protein and Neutrophil Count are highly significantly and clinically correlated.

#### 5.26.4 Confirmed osteomyelitis

Neutrophil Count was the variable in the regression that was considered to be most likely to in the model when antibiotics were not used, it can be seen to be highly statistically and clinically correlated with C Reactive Protein.

### 5.27 Correlation and relationship to other studies about inflammatory markers

This section will attempt to show any relationship between previous studies about inflammatory markers in diabetic foot disease and the correlation seen here.

#### 5.27.1 Correlation and the marker of Neutrophil Count

The inability of Neutrophil Count to predict diabetes associated foot infections as suggested by Pittet *et al* (2008) although based on in – patients maybe in part explained by the statistical and clinical correlation with C Reactive Protein and to a lesser statistical and clinical degree (but still significant) Plasma Viscosity in cutaneous infection and C Reactive Protein in both suspected and confirmed osteomyelitis in this study.

#### 5.27.2 Correlation and the marker of Haemoglobin

The study on in – patients by Upchurch *et al* (1997) suggested diabetes and foot infection was likely to reduce Haemoglobin. The negative correlations seen here with all wounds with infection may support this.

#### 5.27.3 Correlation and the marker of C Reactive Protein

C Reactive Protein is consistently and statistically significantly ( $p \leq 0.001$ ) correlated with Neutrophil Count across this study. In clean wounds the Pearson correlation coefficient is 0.666, in cutaneous infection 0.513, in suspected osteomyelitis 0.582, and in confirmed osteomyelitis 0.629. The effect of diabetes as a disease on C Reactive Protein is not fully explained in relation to foot disease by Nesto (2004). The suggested rise caused by diabetes alone may in part explain the association with Neutrophil Count which from the healing process of a wound would be expected to be raised.

#### 5.27.4 Correlation and the marker of Plasma Viscosity

Plasma Viscosity, if associated with neuropathy as put forward by Mac Rury *et al* (1990), may be expected to be more strongly correlated with the other markers and this is not seen.

#### 5.27.5 Correlation and the marker of Procalcitonin

Procalcitonin shows only one correlation and that is with Neutrophil Count in clean wounds. In this situation the expected level of Procalcitonin would be very low as would the level of Neutrophils as they prime function of this cell group is bacterial ingestion – not necessary in a clean wound.

## **Chapter 6 Methodology The use of SPECT/CT in the diagnosis of osteomyelitis**

This chapter will provide an outline of the method of the second part of the study in which the use of Single Photon Emission Computed Tomography / Computed Tomography (SPECT/CT) scans were compared to a reference test of Magnetic Resonance Imaging (MRI).

### **6.1 Research question**

Is SPECT/CT a useful diagnostic tool for recognising osteomyelitis in patients with diabetic foot ulcers?

### **6.2 Research aim**

To determine the sensitivity and specificity of SPECT/CT in the diagnosis of osteomyelitis in diabetic foot ulcers compared to MRI as the gold standard.

### **6.3 Research objective**

To compare the diagnostic accuracy of SPECT/CT in recognising osteomyelitis in diabetic foot ulcers against MRI.

### **6.4 Protocol**

The use of an alternative scanning technique for the diagnosis of osteomyelitis associated with diabetic foot ulcers would be very useful and this research was conducted to determine if the new technique of SPECT/CT is comparable in the results it produces to MRI.

## 6.5 Summary

This study investigates the potential use of the imaging technique of Single Photon Computed Tomography / Computed Tomography in making a diagnosis of osteomyelitis in foot ulcers associated with diabetes.

## 6.6 Keywords

Imaging Diagnosis Osteomyelitis

## 6.7 Rationale of method

MRI is the most commonly used method to diagnose osteomyelitis within the usual practice of the diabetic foot team in the Royal United Hospital, Bath. Experienced musculoskeletal radiologists read the scans ensuring that continuity and as such inter and intra rater reliability is maintained. The use of MRI to confirm suspect osteomyelitis continues to be the confirmatory diagnostic tool. However not all individuals are able to undergo the MRI procedure for a number of reasons including claustrophobia and metal implants. The use of another form of three dimensional imaging that can determine the extent of infection would be most useful. The additional anatomic data provided by the Computed Tomography element of the SPECT/CT may suggest an improvement in the diagnostic sensitivity of the usual nuclear medicine bone scan. The advancement is made by the combination of the standard technique of nuclear imaging with computed tomography to provide this option. This use in diabetic foot infection detection is a new development for this technique. The experienced consultant radiologist involved in the nuclear medicine department was used as the constant reader of the scans (as for the MRI s being read by the same individual) to ensure a consistent inter and intra rater reliability was maintained.

#### 6.7.1 Imaging techniques used

The many different imaging techniques available are discussed in more depth in the introduction. The rationale for the choice of the reference test is also discussed.

MRI was chosen as the reference test in this study to assess the clinical utility of SPECT/CT against. Each of the techniques used in this reported study will be discussed in greater depth.

##### 6.7.1.1 Magnetic Resonance Imaging

MRI reveals active medullary osteomyelitis as an area of abnormal marrow with altered signal. It is this that is looked for when the scan is read with the possibility of intraosseous collections. The nature of the scan producing slices of image allows localisation of any defect.

##### 6.7.1.2 Single Photon Emission Computed Tomography /Computed Tomography

The changes that occur in this scan that indicate a pathological process are uptake of the tracer within the bones that is a result of the increased blood flow to the diseased areas. The computed tomography element allows localization of the diseased area by the acquisition of a three dimensional image of the foot by producing slices of the image in body planes.

#### 6.8 Imaging and the disease of diabetes

Diabetes can cause problems with the imaging. This is from the fact that concurrent renal disease can reduce the capacity for an individual to clear any contrast medium that is used to enhance the images produced. This is particularly true for the Gadoteric acid as used in MRI. The obesity of some patients with in particular type 2 diabetes, makes them unsuitable for the MRI scanner in Bath as it is enclosed. On

occasion there has been the capacity to perform the scan by the patient being 'reversed' and only the lower limb entering the data acquisition area.

## 6.9 Confounding factors

The presence of Charcot neuroarthropathy (another complication associated with diabetic foot disease) can cause diagnostic difficulties when looking for a diagnosis of infection (Tan and Teh 2007). This is the case for all imaging techniques.

### 6.9.1 Recognition and Managing of confounding factors

In an attempt to limit the uncertainty caused when Charcot is present no patients with active Charcot joints (recognized clinically by abnormal skin temperatures taken using an infra-red thermometer and deformity of the foot) were enrolled into the study.

Individuals that had both scans were booked for the imaging as close together as practical to avoid changes in the disease process and avoid the second scan which ever that was being influenced by the results of the first being known.

The analysis of image results is by its very nature a subjective analytical technique using human beings to read results from what their experience tells them they are seeing within the scan. In an attempt to reduce the variability of this the Consultant Radiologist with a specific interest in Musculoskeletal imaging for the Magnetic Resonance Imaging and likewise for Nuclear Medicine imaging for the Single Photon Computed Tomography / Computed Tomography imaging agreed to report the results. This was to make certain that the more experienced clinicians were looking at the scans and as reading a scan has some level of subjectivity this was reduced.

## 6.10 Sample

Individuals were approached that attended the multiprofessional Diabetes Foot Clinic in an acute district general hospital. The hospital is a 687 bed hospital covering geographical area that is a mixture of rural and urban with some towns and villages, having a population of some 550000. Within the catchment area there are community hospitals and Minor Injury Units. The individuals had been referred for specialist care advice and treatment of their foot ulcer to the diabetes team by community podiatrists, practice and district nurses and General Practitioners. The Diabetes Foot Clinic team consist of a consultant diabetologist with a special interest in feet, a consultant vascular surgeon with a special interest in diabetes and feet, two experienced podiatrists, an orthotist all with rapid access to a consultant microbiologist with a special interest in infectious diseases, consultant dermatologists, consultants in orthopaedics, vascular technicians and plaster technicians.

They agreed with formal written consent to take part. The individuals were those in whom the usual clinical care in Bath would be to have a referral for an MRI scan due to unsure diagnosis.

The use of broad non specific criteria for the recruitment process was an attempt to recruit as many individuals as possible without including individuals who

- could be seen to be requiring immediate emergency care for the presenting problem,
- to be seen to potentially been coerced into partaking,
- to have a disease different to diabetes that was likely to affect the inflammatory markers and that had the potential to be harmed by the process e.g. the exposure to an unborn child of the radiation in the Single Photon Computed Tomography / Computed Tomography part of the study.

### 6.11 Method; Ethical statement

Approval from the relevant NHS trusts and from both the University of Bath and COREC ethics system was sought and followed. This ensured that ‘good clinical practice’ was maintained throughout, and either taking part or refusal did not compromise clinical care for that individual or any others. Research governance procedures including securing Research and Development (R&D) approval was also performed. The storage of patient sensitive information was also on a NHS computer that was sign – on and password protected with additional passwords to access any research material.

### 6.12 Method; Ethical considerations

Exposure to radiation through the process of the SPECT/CT scan was explained in the verbal explanation of the research and was included on the patient information sheet. This was described in a manner that made the degree of exposure understandable – likening it to living in the county of Cornwall for 33 days. Additional information about both scans was provided by the relevant departments. The additional time taken for the scan was explained and so was the need to return to the department several times for scans at different stages over the course of several hours but the ability to ‘come and go’ as they wished between scans.

Discussions between the researcher and radiology and nuclear medicine departments took place to ensure that the research patients did not compromise the waiting of ‘ordinary’ patients for the scans.

Parking permits were only available to those individuals who were asked to undertake the additional scanning technique as this involved an additional attendance above normal clinical expectations.



The confidentiality required in obtaining the participants personal information from the diabetes data base was covered in the course of normal clinical practice by the Caldicott Guardian and Data Protection principals.

All information was stored on a NHS computer user name and password protected. The results of the imaging techniques were further user name and password protected on a web based image capture system.

The data was anonymised within the results database.

### 6.13 Inclusion / exclusion criteria

#### INCLUSION

- to have a diabetes related foot ulcer
- to be able to give informed consent
- to be able to have both MRI and SPECT/CT scans

#### EXCLUSION

- to have overwhelming sepsis requiring immediate medical and/or surgical treatment
- to be unable to give informed consent
- to be unable to have MRI scan for example having a metal implant or due to claustrophobia (or MRI restriction by patient size)
- to have a reduced renal function that prevents the use of contrast medium in the imaging processes
- to have a concurrent disease likely to affect inflammatory markers e.g. rheumatoid arthritis
- to be pregnant
- to be breast feeding.

## 6.14 Consent

Individuals were given the patient information sheet as they reported to reception in the Diabetes and Endocrinology centre for the Diabetes foot Clinic to allow for some time to read the information prior to being approached taking part in the research.

The patient information leaflet had been accepted by the ethics committee as having the wording of a certain size and font to assist legibility and also in a language suitable for lay individuals.

The patient information sheet asked individuals to consider giving their consent for the researcher for both the research, i.e. venepuncture immediately and referral for imaging where deemed appropriate. Not all individuals partook in both parts of the study.

The care the individual was booked into the clinic to receive was provided prior to any discussion about the research.

Information about the method of using the different scanning techniques was provided in clinic to anybody consenting to the use of the two different techniques. Examples of this are the length of time the appointment was likely to last and the degree to which they would be exposed to radiation as the term nuclear medicine (the department where the Single Photon Computed Tomography / Computed Tomography scan takes place) was alarming to some. Both the radiology and nuclear medicine departments sent out further information about the scans with the appointment bookings.

## 6.15 Method of study

### 16.15.1 Using MRI as the reference test

Magnetic Resonance Imaging (MRI) is used commonly as the gold standard imaging technique in the United Kingdom to recognise infection is because it presents both a detailed accurate image including areas of soft tissue oedema and bone abscesses (Mader, Cripps and Calhoun 1999). Sartoris (1994) has performed a meta analysis on ten previous studies to assist recognition of abnormal soft tissue signals with MRI. It is also more sensitive and precedes these changes than plain film or simple Computerised Tomography (Crim and Seeger 1994). MRI can be used to give good structural visualization and spatial resolution (Paluska 2004), superior contrast resolution and a multiplanar examination (Flemming, Murphey and McCarthy 2005). Reported sensitivities are high ranging from 88%-100% with a specificity of 53%-94% (*ibid*). It is recognised that specialist technical and interpretive skills are required to achieve these levels (Berendt and Lipsky 2004). This puts forward the suggestion of another test, for example the three dimensional scanning technique Single Photon Computed Tomography / Computed Tomography, being used in addition.

Another meta-analysis of MRI studies has shown that the procedure performs well in the diagnosis of osteomyelitis of the foot and ankle in adults (Kapoor *et al* 2007). This study is good in that it acknowledges the problems associated with the combination of the studies. These include using English language only articles with few studies following the assessment of individuals with scan results read blind to other diagnostic techniques or with biopsy as a confirmatory tool. The presence or absence of Charcot foot was not typically documented and it is recognised that this is one of the potential differential diagnoses particularly in the diabetic population. The advancements made in the MRI technique are also acknowledged as possible ways of diagnosis being made with greater certainty in the more recent studies, for

example the use of Gadolinium as a tracer and the use of secondary diagnostic signs such as cortical breaks.

## 6.16 Methodology

The usual practices of referral for MRI was used when clinical suspicion of deep infection was present without any positive suggestion of either abscess or bone infection. This was then also performed using the SPECT/CT scan.

SPECT/CT is a new method of combining nuclear medicine techniques of bone scanning with computerised tomography to localise any bony abnormality found. To recognise if SPECT/CT will be of benefit in diagnosis of osteomyelitis especially in these patients, each patient will also have both a MRI and a SPECT/CT scan. The SPECT/CT results will be compared to those of the MRI. The additional amount of radiation exposure in delivering the CT element of the scan is less than 0.1 mSv. In lay terms this is the equivalent of living in Cornwall for five days. The amount of radiation to which an individual was exposed during such a scan was controlled by the Medical physics department. The administration of the radioisotope was using Diphosphonate labelled to 99mTechnetium, equivalent to 600 Milli Becquerels. The trade name for this being HMDP®. The clinical effectiveness will be measured by comparing diagnostic rates of MRI to SPECT/CT.

Written additional information about both forms of scans was provided and sent by the relevant departments when booking the patient's appointment.

The request sent to the departments informed them that the patient had diabetes and a foot ulcer, with the anatomic site named, with a query about the presence of deep infection such as abscess or osteomyelitis.

#### 6.16.1 Magnetic Resonance Image acquisition protocol

Due to the nature of the MRI scanning procedure patients were required to remove any metal containing clothing and if necessary to wear a hospital gown.

The standard protocol for imaging suspected osteomyelitis in the foot was performed. This involves the following sequence of image attainment;

T1 axial

Stir axial

Proton density fat saturated axial

STIR coronal. (STIR being the acronym for short-tau inversion-recovery sequences when water is 'bright' on the images the inverse of the usual fat 'brightness').

Followed by the administration of contrast medium and sequence;

T1 axial

T1 coronal.

These are through the axial and coronal body planes. The effect is to produce 'sliced' images across the width of the foot (axial) and along the length of the foot (coronal) allowing accurate localization of any abnormality. The slice width being of the order of 3-4 mm.

The contrast medium used was Dotarem® made by Guerbet. This has Gadoteric acid as its active ingredient formed in situ from DOTA and gadolinium oxide at an equivalent level of 0.5mmol/ml. The dose of drug given being calculated by body mass at 0.2ml per kilogram mass. Administration is by the use of calibrated pre-filled syringes into a vein. It is the gadolinium oxide that is the magnetic agent and enhances the images produced.

#### 6.16.2 Single Photon Computed Tomography / Computed Tomography Image acquisition protocol

The SPECT/CT scanning procedure allows an individual to remain fully clothed providing access to a vein is possible for injection of the tracer material.

A standard 3 phase bone scan was performed followed by SPECT/CT images of the feet. The standard scan involves the patient being injected intravenously with Diphosphonate labelled with 99mTechnetium to a level of 600Milli Becquerels. This is a standard dose that is only altered very occasionally dependant on body mass. For all images patient lies supine on scanning table. All images are obtained using a double head Infinia gamma camera with Low Energy High Resolution collimators.

The bone scan involves;

- 1<sup>st</sup> phase –with immediate images obtained of the feet (16 frames at 3 second intervals)
- 2<sup>nd</sup> phase – 2 minutes post injection - static blood image of feet obtained (the images are taken to a total of 200000 radioactive ‘counts’ to provide a ‘good’ image)
- 3<sup>rd</sup> phase – anterior & posterior whole body scan performed 3 hours post injection. Scanning from head to toes.

Exposure time per image – 240secs with a speed of travel of scanner 10cm/minute. The result of this is a whole body scan takes approximately 30 minutes with an additional 20 minutes imaging of the feet.

The SPECT/CT images of feet are then obtained using the following protocol;

- Matrix size 128x128, which is the size of image used and is a compromise between image clarity and ‘noise’ (unwanted image detail).

- Slice step 5-10mm, which is images are taken at 5 – 10 mm intervals throughout the image acquisition. The movement of the cameras is through 60 degrees each 30 seconds within a total angular range of 360 degrees.

### 6.17 Diagnosing Osteomyelitis in a MRI scan

As described by the diagnostic papers by Yuh *et al* (1989), Weinstein *et al* (1993) and Ertugrul *et al* (2006) the diagnostic method for identifying osteomyelitis associated with a diabetic foot ulcer the criteria used for positivity were ‘decreased or low signal intensity on T1 weighted images in areas of bone marrow with focal enhancement after the administration of contrast medium and increased signal intensity on STIR and T2 weighted images’. These were also recognised by Dinh, Abad and Safdar in 2008. Rozzanigo *et al* (2008) describe this as a primary sign of evidence. In addition the presence of a ‘cortical breach or intraosseous abscess may also indicate osteomyelitis’ (Morrison *et al* 1995). Secondary signs are described by Rozzanigo *et al* (2008) as being ‘identified close to the altered bone marrow signal and include oedema caused by septic inflammation (cellulitis or phlegmon), soft tissue abscess, skin ulcer and fistula, with possible interruption of the cortical bone’. Yuh *et al* (1989) recognize the clarity offered in the T1 weighted images. The use of contrast media such as gadolinium has been suggested, using the analysis of case studies, by Morrison *et al* (1998) and Tan and Teh (2007) to offer no increase in accuracy of diagnosis of osteomyelitis but to improve the diagnosis of soft tissue pathology.

### 6.18 Diagnosing Osteomyelitis in a SPECT/CT scan

As described by the diagnostic paper by Yuh *et al* (1989) the diagnostic method for identifying osteomyelitis associated with a diabetic foot ulcer the criteria used for positivity were ‘increased blood flow and blood-pool activity and abnormally increased intensity localized to the bone’. The presence and evaluation of cortical

erosions, focal areas of lucency and sequestra has also been noted by Teh, Berendt and Lipsky (2010).

This method of confirmation was originally described for a 3-phase bone scan but is equally applicable to a SPECT/CT scan.

#### 6.19 Analysis of imaging results

The analysis of the written reports was performed by comparison of the wording describing three areas of the two scan reports for each individual. These areas were the anatomical site of concern; with appropriate detail, the detail in which the surrounding tissues were described, and the overall conclusion.

Each of the reports was provided by the relevant medical consultant within their specialty, i.e. musculoskeletal Magnetic Resonance Imaging or musculoskeletal Nuclear Medicine. The reports were made 'blind' to the other scan results.



## **Chapter 7 Results The use of SPECT/CT in the diagnosis of osteomyelitis**

This chapter will provide information gained from the reports of the two scanning techniques that were compared in this research along with the clinical diagnosis or suspicion for each patient. Two patients consented to having both forms of scans but withdrew consent after the MRI had been performed before the SPECT/CT was performed.

### **7.1 Sample characteristics**

The individuals who underwent scans all had a clinical suspicion of osteomyelitis. There were 3 females and 7 males with an age range of 39 - 80 years, mean 61 years. The range and mean duration of diabetes was 1 - 30 years, mean 15 years.

None reported any adverse complication or event of having either scan.

The scans were compared by searching the written reports for commonality in the three areas of site of problem the detail provided and overall conclusion.

## 7.2 Scan results

Table 7.2.1 Patient 1

clinical presentation/ suspicion - Deep or bone infection and / or new Charcot process on top of old in right mid foot

	MRI	SPECT/CT
Site;	Mid foot, tarsal bones, calcaneum – posterior subtalar joint	Calcaneo talar and talo navicular joints
Detail	Oedema, cyst formation, 7mm diameter abscess	Very aggressive destruction of joints
Conclusion	Suspicious for intraosseous abscess in calcaneum	Suspicious for osteomyelitis
Agreement with clinical presentation	Yes	Yes

Table 7.2.2. Patient 2

clinical presentation / suspicion – abscess an / or osteomyelitis right 2<sup>nd</sup> metatarsal head

	MRI	SPECT/CT
Site;	2 <sup>nd</sup> metatarsal phalangeal joint	Mid right fore foot,
Detail	Proximal phalanx of 2 <sup>nd</sup> toe, discontinuity of FDL tendon	Distal ½ 2 <sup>nd</sup> metatarsal
Conclusion	Highly convincing for Osteomyelitis	Osteomyelitis of distal right 2 <sup>nd</sup> metatarsal
Agreement with clinical presentation	Yes	Yes

Table 7.2.3 Patient 3

clinical presentation / suspicion – right heel infection probably cutaneous only

	MRI	SPECT/CT
Site;	Calcaneum	Calcaneum and cuboid, navicular and base of 4 & 5 metatarsal
Detail	Tiny fluid collection	Suggestive of infection
Conclusion	In keeping with cellulitis, possibly an area of osteomyelitis	Osteomyelitis of Calcaneum and cuboid with probable involvement of the navicular and base of 4 & 5 metatarsal
Agreement with clinical presentation	Yes	Yes

Table 7.2.4 Patient 4

clinical presentation / suspicion – osteomyelitis of right heel

	MRI	SPECT/CT
Site;	Right heel	Right hind foot, right calcaneum
Detail	Subcutaneous collection 2.2cm diameter, horizontal cleavage of the os calcis with elevation of the upper half	Medial anterior aspect of calcaneum
Conclusion	Not possible to tell if infective or not	Acute infection and/or healing trauma
Agreement with clinical presentation	No	Yes

Table 7.2.5 Patient 5

clinical presentation / suspicion – osteomyelitis of left hallux

	MRI	SPECT/CT
Site;	Terminal phalanx big toe	Left great toe metarsophalangeal joint
Detail	1 <sup>st</sup> metatarsal head, 1 <sup>st</sup> MTPJ and proximal phalanx of the big toe, area of non enhancement in 1 <sup>st</sup> proximal phalanx measuring 1.4x0.5x0.5 cm consistent with a focus of osteomyelitis / osseous abscess, thickening of flexor hallucis tendon	Head of the left great toe metatarsal extending into the left great toe
Conclusion	Deep ulceration below the 1 <sup>st</sup> MT and MTPJ with underlying osteomyelitis	Osteomyelitis
Agreement with clinical presentation	Yes	Yes

Table 7.2.6 Patient 6

clinical presentation / suspicion – osteomyelitis of left first metatarsal head

	MRI	SPECT/CT
Site;	1 <sup>st</sup> MTP	Right great toe metatarsal head
Detail	Sinus track running from soft tissue towards the joint, several small pockets of fluid/ abscesses	Extends from the head of the metatarsal into the proximal phalanx of the right great toe, superior and inferior aspects
Conclusion	Consistent with Osteomyelitis of 1 <sup>st</sup> metatarsal and proximal phalanx	Osteomyelitis or septic arthritis although this is not entirely specific
Agreement with clinical presentation	Yes	Yes

Table 7.2.7 Patient 7

clinical presentation / suspicion – Deep infection likely osteomyelitis with severe cutaneous infection of right fifth toe

	MRI	SPECT/CT
Site;	4 <sup>th</sup> and 5 <sup>th</sup> metatarsal heads	Head of right 5 <sup>th</sup> metatarsal and proximal phalanx of 5 <sup>th</sup> toe
Detail	Abscess between the 4 <sup>th</sup> and 5 <sup>th</sup> metatarsal heads extending to the level of the middle or distal phalanx of the little toe 3cmx1cm, proximal phalanx enhances suggesting infection	right 5 <sup>th</sup> metatarsal head and proximal phalanx, not extending significantly up the shaft of the metatarsal
Conclusion	The major abnormality is in the soft tissue but it is likely the little toe proximal phalanx is involved by osteomyelitis with septic arthritis	Bone scanning alone would suggest infection however the differential includes an intense inflammatory arthropathy
Agreement with clinical presentation	Yes	Yes

Table 7.2.8 Patient 8

clinical presentation / suspicion – left deep heel infection, osteomyelitis

	MRI	SPECT/CT
Site;	Os calcis	Left heel
Detail	Concave defect 2 x 1.8 x 1 cm and oedema extending into the body of the calcaneum	Postero lateral aspect of the left calcaneum
Conclusion	Osteomyelitis	Osteomyelitis
Agreement with clinical presentation	Yes	Yes

Table 7.2.9 Patient 9

clinical presentation / suspicion – osteomyelitis of right distal hallux

	MRI	SPECT/CT
Site;	Right hallux	Right big toe
Detail	There is soft tissue swelling and oedema, especially of the hallux. No soft tissue collections, There is some marrow oedema in the phalanges of the hallux but no suggestion of intra osseous collection. The marrow oedema could be reactive and thee is no evidence of complications of osteomyelitis.	Markedly increased tracer flow within the right foot ..... Within the right big toe on the blood pool and delayed images
Conclusion	Extensive inflammatory change but no MRI evidence of osteomyelitis.	Right big toe bone infection
Agreement with clinical presentation	No	Yes



Table 7.2.10 Patient 10

clinical presentation / suspicion – cellulitis to mid foot with apical ulcer 2<sup>nd</sup> toe complicated by ?osteomyelitis?

	MRI	SPECT/CT
Site;	Terminal phalanx second toe	Tip of left second toe
Detail	The terminal phalanx of the second toe has low signal in its marrow and this does not enhance suggesting bone necrosis. The oedema of the proximal and middle phalanges does enhance, showing perfusion. No soft tissue fluid collection is seen.	There is marked increased blood flow and mild increased uptake in the tip of the left second toe.
Conclusion	Bone necrosis with infection.	The findings in the left second toe could represent osteomyelitis although are not entirely specific.
Agreement with clinical presentation	Yes.	Yes.

## **Chapter 8 Discussion The use of SPECT/CT in the diagnosis of osteomyelitis**

This chapter will provide a discussion about the two forms of imaging used in this study and the clinical utility of both. There was however insufficient numbers of scans performed to make any meaningful measure of specificity and sensitivity.

Both tests in this series were influenced by the predictive value in that it was influenced by the underlying prevalence of disease. Review bias was eliminated as far as was practical and using the same expert practitioners to read the scans reducing the subjective element of reading a scan.

From a medical view it is necessary to be able to evaluate the extent of both soft tissue and bone involvement (Berendt *et al* 2008). This determines the need for antibiotic therapy and all the decisions that encompasses and the need for referral to surgery.

From a surgical view again it is necessary to be able to evaluate the extent of both soft tissue and bone involvement to assist in surgical planning (Berendt *et al* 2008).

Only one paper has been found discussing the use of computed tomography and not specifically SPECT/CT in the diagnosis of foot infection for comparison with the results of this research. In most the use of bone scans is discussed but not with the additional Computerised Tomography element. MRI is well documented as a

diagnostic tool in examining the foot in diabetes for determination of the presence and / or absence of bone infection.

Prior to the introduction of SPECT/CT CT was limited by the small surface area involved in foot infections and lack of multi planar activity (Weinstein *et al* 1993). The images that can now be produced allow viewing in all body planes and as such allow a greater amount of detail for precise positioning of pathological disease to be recognised. This was previously only seen within the images of MRI.

The criteria used for a positive diagnosis of osteomyelitis within this study were the same as those proposed by Yuh *et al* (1989), Weinstein *et al* (1993), Ertugrul *et al* (2006) and Dinh, Abad and Sadfar (2008). Namely when using MRI ‘a decreased signal intensity on T1 images with focal enhancement after contrast and increased signal intensity on T2 weighted images’. When using bone scintigraphy a positive result for the diagnosis of osteomyelitis was used when ‘an increased blood flow and blood-pool activity and abnormality increased intensity localised to the bone’ (Yuh *et al* 1998) was present.

The diagnostic signs of osteomyelitis with MRI require some experience to recognise them and to interpret the results and help in the difficult differential diagnosis of infection and Charcot neuroarthropathy. A consideration that may be used as a sign diagnostic of osteomyelitis by Tan and Teh (2007) in MRI imaging is the visualization of abnormal signal and enhancement extending from the skin to a bone in question. Other features of images suggested by Ahmadi *et al* (2006) that may assist in the differential diagnosis on MR that may become more evident with greater use of SPECT/CT are sinus tracts, replacement of the soft tissue fat, fluid collections and extensive marrow abnormalities. These changes are clearly not as easy to consider in the images produced by SPECT/CT imaging although it may become so with more experience of reading the scans. Teh, Berendt and Lipsky (2010) suggest the soft tissue contrast in computed tomography is poor compared to

Magnetic Resonance Imaging but offer no more clarity as to why or how it may be improved.

SPECT/CT has three advantages; in that as the scanning method is the patient is not required to be enclosed by the data acquisition head (it moves around the patient) there is less concern for the morbidly obese patient as many patients with diabetes are and as such unable to fit within the MRI scanner, there is no impact should there be metal within the patient e.g. a foreign body within the eye or a joint prosthesis, and no contrast that is likely to effect renal function is required to enhance the images; important in many patients with diabetes and established renal disease. If an increased determination of sinus tracks and or soft tissue abscesses contrast can be administered but is generally not required for the recognition of bone disease. The need to administer a small amount of radioactive material to the individual patient maybe seen as a disadvantage to some. The problem that may cause SPECT/CT to be less sensitive is the diabetes complication of ischaemia reducing the peripheral uptake of the radioactive marker; the diphosphonate distributes in an amount proportional to blood flow. However as the result is a comparison of 1) both limbs as opposed to an absolute and 2) the relative uptake within the affected limb it may be this is not clinically important.

Both imaging modalities appear to give the information required to determine the presence or absence of bone disease and the extent to which this affects the foot as a whole system. So in conclusion SPECT/CT seems a reasonable alternative to MRI where this is either not available or not suitable.

A general discussion of the diagnosis of osteomyelitis follows in the next chapter. This includes mention of the problems that need addressing in any future study.

## **Chapter 9 General Discussion, Recommendations and Conclusions**

Osteomyelitis remains a challenge to diagnose or exclude as the many tests available provide ambiguous results (Lipsky *et al* 2004). It has been suggested that unexplained inflammatory markers should arouse suspicion of bone infection (Kaleta, Fleischli and Reilly 2001) with no clear guidance as to what a raised level is for most of the markers commonly used. It is given the strength of evidence as B – moderate evidence to support and the standard of evidence as II – evidence from  $\geq 1$  well designed clinical trial without randomisation; from cohort or case controlled analytic studies (preferably from  $>1$  centre). Of the markers I have measured and reported here it can be seen Neutrophil Count is at the high end of the accepted normal range, C Reactive Protein is raised above the accepted normal range, Plasma Viscosity is raised above the accepted normal range and Procalcitonin is raised above the normal level in osteomyelitis but with no clear pattern in any marker, or group of markers, that could be used as a diagnostic test.

The many confounding factors within wound care make any study difficult to interpret and provides limited broad applicability. It was hoped that a combination of blood tests for inflammatory markers may have shown some positive indication of osteomyelitis. This has not proved possible; in this study the participants were neither new presentations and as such having the potential to be antibiotic naïve or were ill enough to require admission to hospital. Many of the previous studies have been used on in - patients and some have been able to enrol antibiotic naïve individuals, this has limited the comparability to other work.

The results presented illustrate a lack of clarity when using inflammatory blood markers to assist in the diagnosis of any infection in diabetic foot ulcers. Major pathogens have been shown to adhere to bone by expressing adhesion factors for components of bone matrix (Hartmann-Heurtier and Senneville 2008) and this may

be a partial explanation for some of the unexpected results. The infective bacteria in this scenario being 'hidden' from the immune response may reduce the degree of inflammation produced. The inflammation is the start of the defence system against infection. The inability to see a clear pattern within the blood markers to recognise either cutaneous infection or osteomyelitis was disappointing. Any future study that uses inflammatory blood markers needs to consider the data gathering in an attempt to recognise and allow for confounding factors. The confounders not considered in this research include making any attempt to both define and stratify into groups (using cluster sampling and analysis), the chronicity of the wound and, where appropriate, the same for osteomyelitis. The virulence of the causative micro organism in infection may also be of interest. The nature of the disease process of diabetes alone confounding the results makes it complex without the addition of potential confounding from diabetes associated complications and the medications associated with treatment. Any future study may do well to consider these points. Unfortunately these and other confounding factors that could have the potential to affect the results of the blood tests are the reality faced in the out patient diabetic foot clinic.

Currently there is insufficient evidence to consider any change in current practice. The use of the inflammatory markers alone for diagnostic purposes has not been seen to be possible and as such they should continue to be used alongside clinical signs, symptoms and judgement to determine the extent of disease.

Imaging studies continue to help diagnose or better define deep, soft tissue purulent collections and detecting pathological processes in bone (Lipsky *et al* 2004). SPECT/CT looks promising for those individual unable to have a MRI through reasons including obesity, renal impairment and metallic implants. The diagnostic accuracy from the limited cases presented here appears promising. Greater experience in the reading of SPECT/CT scans in relation to the diagnosis of osteomyelitis will assist in the diagnostic features seen in this form of imaging.

In terms of the imaging study it would be beneficial to have a wider range of conditions examined and a greater number of individuals included.

SPECT/CT relies on blood flow (and pooling) increasing to allow recognition of the area of pathological change. In this study the inflammation initiated by the infective process causes the increased blood surge. The additional CT element to previous nuclear medicine bone scanning techniques does not appear to have increased the specificity in localising the pathology to skin and or bone. However, nor has it provided the precise detail MRI can offer. This may improve with the development of the skills of the staff concerned in the recognition of normal and abnormal findings in the foot.

The most appropriate method of diagnosing osteomyelitis with the most robust technique, in suitable individuals, would appear to be an image guided percutaneous bone biopsy through skin adjacent to the ulcer. This reduces the risks of contamination from cutaneous and other superficial micro organisms and can allow the collection of the causative micro organisms in the bone. Clearly the problems of introducing a further wound are far from ideal in this population, and the risks and benefits of creating another wound must be considered carefully.

As a second line of diagnosis it would appear practical to consider MRI when used with Gadoteric Acid to enhance the images. Using highly skilled staff in reading the scan is also essential. This is the ideal situation where the individual does not have renal impairment which many foot clinic patients do have; renal disease has the capacity to affect the ability to excrete the contrast drug. A non-enhanced MRI can also be beneficial when evaluated by a highly skilled radiologist.

SPECT/CT continues to have initial problems with producing the precise definitions that MRI is capable of for soft tissue and the accurate sizes of bony defects. These may well reduce as the technique is used more and the clinicians involved learn more about the functionality it provides in diagnostic procedures in the lower limb and foot in particular.

Until these problems are overcome osteomyelitis associated with diabetes will remain a condition which is difficult to recognise and in which treatment often fails, with the end result of an amputation to remove the infected tissues.



## **Chapter 10 Appendices**

### **10.1 Ethics approval**



**Bath Local Research Ethics Committee**

Room 11, John Apley Building  
Research Ethics Office  
Royal United Hospital  
Combe Park  
Bath  
BA1 3NG

Tel/Fax: 01225 825725

vanessa.bishop@ruh-bath.swest.nhs.uk

12 January 2007

Miss Kim Harman  
Lead Podiatrist Diabetes  
Diabetes Centre  
Royal United Hospital  
Bath, BA1 3NG

Dear Miss Harman

**Full title of study:** Can we improve how we diagnose osteomyelitis in the diabetic foot?  
**REC reference number:** 06/Q2001/216

Thank you for your letter of 21 December 2006, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

**Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

**Ethical review of research sites**

The Committee has designated this study as exempt from site-specific assessment (SSA). There is no requirement for other Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

**Conditions of approval**

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

**Approved documents**

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Application	5.2	08 November 2006
Investigator CV		08 November 2006
Protocol		08 November 2006

Covering Letter		08 November 2006
Summary/Synopsis	1	08 November 2006
Letter from Sponsor		30 October 2006
Peer Review		17 July 2006
Peer Review		30 October 2006
Compensation Arrangements		02 November 2006
GP/Consultant Information Sheets	1	08 November 2006
Participant Information Sheet	2	21 December 2006
Participant Consent Form	1	08 November 2006
Response to Request for Further Information		21 December 2006
Letter from Andrea Taylor re peer review		20 December 2006
Dose calculation		02 November 2006
CV Dr A Robinson		19 October 2006
CV Dr G Taylor		08 November 2006

#### Research governance approval

You should arrange for the R&D department at all relevant NHS care organisations to be notified that the research will be taking place, and provide a copy of the REC application, the protocol and this letter

All researchers and research collaborators who will be participating in the research must obtain final research governance approval before commencing any research procedures. Where a substantive contract is not held with the care organisation, it may be necessary for an honorary contract to be issued before approval for the research can be given.

#### Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

06/Q2001/216

Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

*Vanessa Bishop*

 Dr Brian Robinson  
Chair

Enclosures: Standard approval conditions [SL-AC1 for CTIMPs. SL-AC2 for other studies]

Copy to: Dr Andrea Taylor, Course Director, School for Health, Norwood House, Claverton Down, Bath, BA2 7AY

Dr Alistair Taylor, R&D Manager, RUH

## 10.2 Ethics approval for amendment



## National Research Ethics Service

### Bath Research Ethics Committee

Room 11, John Apley Building  
Research Ethics Office  
Royal United Hospital  
Combe Park  
Bath  
BA1 3NG  
Tel/Fax: 01225 825725  
Research.ethics@ruh-bath.swest.nhs.uk

14 February 2008

Miss Kim Harman  
Lead Podiatrist Diabetes  
Podiatry  
Diabetes Centre, Royal United Hospital  
Bath  
BA1 3NG

Dear Miss Harman

**Study title:** Can we improve how we diagnose osteomyelitis in the diabetic foot?  
**REC reference:** 06/Q2001/216  
**Amendment number:** 3.1  
**Amendment date:** 05 February 2008

The above amendment was reviewed at the meeting of the Sub-Committee of the REC held on 12 February 2008.

#### Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

#### Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Protocol		05 February 2008
Participant Information Sheet		05 February 2008
Notice of Substantial Amendment (non-CTIMPs)	3.1	05 February 2008

#### Membership of the Committee

The members of the Committee who were present at the meeting are listed on the attached sheet.



#### **R&D approval**

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

#### **Statement of compliance**

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

**06/Q2001/216:**

**Please quote this number on all correspondence**

Yours sincerely

**Jenifer Lander**  
**Committee Co-ordinator**

E-mail: [jenifer.lander@ruh-bath.swest.nhs.uk](mailto:jenifer.lander@ruh-bath.swest.nhs.uk)

#### **Enclosures**

List of names and professions of members who were present at the meeting and those who submitted written comments

#### **Copy to:**

Dr Andrea Taylor, Course Director, School for Health, University of Bath,  
Norwood House, Claverton Down, Bath BA2 7AY  
Dr Alistair Taylor, R&D Manager, Bath & Wessex House, RUH

**Bath Research Ethics Committee**

**Attendance at Sub-Committee of the REC meeting on 12 February 2008**

<i>Name</i>	<i>Profession</i>	<i>Capacity</i>
Mr Anthony Harrison	Consultant Nurse & Visiting Research Fellow	None
Dr. Gordon Taylor	Research and Development Support Unit University of Bath, Wolfson Centre	None

**Also in attendance:**

<i>Name</i>	<i>Position (or reason for attending)</i>
Miss Vanessa Bishop	REC Co-ordinator

### 10.3 Research and Development approval



# Royal United Hospital Bath

NHS Trust

Research & Development Department

Date: 18/01/07

Dear Miss Harman

Re: REC ref : 06/Q2001/216  
RD ref: 01316

**Project Title: *Can we improve how we diagnose osteomyelitis in the diabetic foot.***

I am pleased to tell you that the above project has now been approved by the Royal United Hospital Bath NHS Trust (RUH). However before you start this project, you must demonstrate to the R&D department that you have also obtained written full approval from the local research ethics committee before the research begins. Our approval is subject to full ethical approval.

It is essential that this project be carried out according to Good Clinical Practice and within the guidelines of the NHS Research Governance Framework for Health and Social Care. Full information about the Research Governance Framework can be obtained via the internet on <http://www.doh.gov.uk/research/rd3/nhsrandd/researchgovernance/govhome.htm>.

Unless you have been given an Investigator research site file by the sponsor or Chief Investigator, you must use an Investigator research site file provided by us, to enable easy research audit. This file can be obtained from the R&D Office in Bath & Wessex House RUH.

You have responsibility for ensuring that all participants sign informed consent and that the protocol agreed by the Research Ethics Committee (REC) is adhered to by yourself and any other co-workers. Please ensure that the version of the protocol and supporting documents held by the Research and Development Office are the same as the ones approved by the REC and provide up-to-date versions if necessary.

May I also remind you that as Principal Investigator or Chief Investigator you will be required to provide us with information concerning safety, monitoring and outcome information for this study, including a lay summary upon completion of the research. Investigators who fail to provide timely information on projects may compromise their ability to obtain Trust approval for future work. An information sheet outlining your responsibilities as Principal Investigator or Chief Investigator is enclosed with this letter.

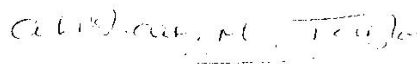
RUH R&D approval for this study is valid until **18/07/08**. Requests to extend the period of approval must be made, in writing, to the Research and Development Office.

Congratulations on initiating this research study. We wish you every success. We are keen to support good research at the RUH and are pleased that you have decided to conduct your study here.

If you need any support or information please do not hesitate to contact the R&D Department; Dr Alistair Taylor, R&D Manager on 01225 824160 or by email ([Alistair.taylor@ruh-bath.swest.nhs.uk](mailto:Alistair.taylor@ruh-bath.swest.nhs.uk)).

NB:  
Please sign the enclosed copy of this letter and return to Dr Alistair M Taylor the R&D  
Department, RUH.

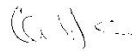
Yours sincerely



Professor John Osborne  
Director of R&D RUH

Dr Alistair M Taylor  
R&D Manager

Researcher's signature:



Cc:

#### 10.4 Research and Development approval following ethics amendment

# Royal United Hospital Bath

NHS Trust

**Research & Development Office**

1st Floor, Wolfson Centre, Royal United Hospital, Combe Park, Bath, BA1 3NG.

**Date: 23/12/2008**

**Dear Miss Kim Harman**

Re: REC 06/Q2001/216  
RD 01316

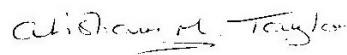
**Project: Can we improve how we diagnose osteomyelitis in the diabetic foot?  
Amendment - 3.1**

I am pleased to tell you that the amendment related to the above named project has been noted and approved by the Royal United Hospital Bath NHS Trust, to recruit patients under the care of this Trust. R&D approval is separate from ethics approval and is also essential for the conduct of research within NHS trusts. It remains subject to the terms of the original approval letter.

If you need any further support or information please do not hesitate to contact us. Congratulations on initiating this project. We are very keen to support good research at the RUH and wish you every success

You will receive two copies of this letter; I would like you to sign both copies keeping one for your own records and one to be sent back to me indicating that you will undertake this research project under the terms stated in this letter. Finally, may I wish you continuing success with your project.

Yours sincerely



**Dr Alistair Taylor,**  
(Research & Development Manager)  
e-mail: [Alistair.Taylor@ruh-bath.swest.nhs.uk](mailto:Alistair.Taylor@ruh-bath.swest.nhs.uk) Tel: 01225-824160

***I agree to undertake this project under the conditions previously laid down and now amended.***

**Signature of Principal Investigator**.....

**Date:**



## 10.5 Patient Information Sheet



## **PATIENT INFORMATION SHEET**

### **Title of Research: Can we improve how we diagnose bone infection in people with diabetes?**

You are being invited to take part in a research study being conducted by Kim Harman, one of the lead podiatrists for Diabetes in the area for a course she is undertaking. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following carefully. Talk to others about the study if you wish.

Ask us if there is anything that is not clear or you would like more information. Take time to decide whether or not you wish to take part.

### **What is the purpose of the study?**

The purpose of the study is two fold:

- 1) to see if certain changes that are known to happen in our blood when we have an injury are different in people with a diabetes related foot ulcer with bone infection to the rest of the diabetes population.
- 2) to find out whether a new form of scan is any better than that we use at the moment.

Diabetes is the most common cause of foot ulcers. These frequently take a long time to heal and often involve infection reaching the bone. Bone infection is presently hard to diagnose and treatment involves antibiotics. Sometimes the antibiotics need to be given by a drip, which is then followed by tablets sometimes for as long as three months.

### **Why have I been chosen?**

You have been chosen because you have diabetes and a foot ulcer. We want to see if there is a difference in those with bone infection related to foot ulcers in a series of blood tests. It is anticipated that a total of 50 people will be approached to partake initially.

### **Do I have to take part?**

You are under no obligation to take part – it is up to you to decide. If you do agree you will be given this information sheet to keep and be asked to sign a consent form. You are free to withdraw at any time up to the point of anonymisation and without giving a reason. A decision not to take part, or withdraw at any time will not affect the standard of care you receive.

If you withdraw from the study up to the point of data being anonymised, we will

destroy all your identifiable samples but will need to use the data collected up to your withdrawal, as it will not be possible because of anonymisation to recognise your individual data.

#### **What will happen to me if I take part?**

If you agree to take part you will be required to have a number of blood tests all of which can be performed by taking 2 samples. This can be done in one session without affecting your health. The research itself will be taking place over the next couple of years. At present not all the blood tests we will be doing on your blood are standard practices in diabetes care; it is whether they should be that we are trying to find out.

You may also be referred to have both a Magnetic Resonance Imaging (MRI) scan and a SPECT/CT scan. (These scans will be if we suspect you have osteomyelitis i.e. bone infection.)

The MRI is the best way we have at present of recognising bone infection. It is a scanning method that uses no radiation, unlike X rays, and will involve an out patient visit of approximately one hour. It is booked and carried out in the radiology department of the Royal United Hospital. The SPECT/CT scan is a new scanning method that involves a very small amount of radioactive material (1ml 1/5<sup>th</sup> a of teaspoon) being injected into your body and a scan some 3 hours later that takes about an hour and a half. You are free to leave the hospital and do as you please during this waiting time. The radiation is equivalent to that you would receive from the background radiation should you spend 33 weeks in Cornwall. This scan is booked and carried out in the nuclear medicine department of the Royal United Hospital.

#### **What do I have to do?**

If you agree please tell a member of staff so that you can go through the consent form and hopefully have the blood taken in clinic today. Alternatively you can go to the 'blood room' within the hospital. If you agree but do not have the time today it may be possible for you to take a blood test form to your GP surgery to have it done at your convenience.

If your ulcer condition changes you may be asked to have further blood tests and possibly additional MRI or SPECT/CT scans.

Blood tests can sometimes be slightly uncomfortable for some people. If you do agree to take part please tell us if you are allergic to tape so we can use a bandage on your arm afterwards.

If in the very rare instance the test shows any problem associated with your



diabetes that the hospital doctors and your GP are not aware of you will be immediately notified by the researcher with a request to see a doctor. The hospital doctors and your GP will be informed immediately of the result, unless you specifically tell us you do not wish to be told if we find any such problem.

#### **Expenses and Payments**

Unfortunately we are not able offer help with travel to, or parking at, the hospital because unfortunately we do not have sufficient funds to allow for this.

#### **What are the side effects?**

Neither scan has side effects and you will be given information about what to wear (no metal) when you receive the appointment

#### **What are the possible benefits/risks of taking part in the research?**

At the moment there is no benefit to you for taking part but it is hoped it will give us more information about the blood changes in people with bone infection associated with diabetes related foot ulcers. If a change is found this may allow quicker recognition of the problem and so more rapid treatment.

#### **How will my taking part in the study be kept confidential?**

If you join the study your confidentiality will be maintained during the study as only the researcher knows of your participation and the results of the blood tests will be stored in a coded manner such that any individual cannot be recognised. Additionally your permission is sought to allow the researcher, and representatives of regulatory authorities (to ensure that the study is being carried out correctly) access to your diabetes healthcare record to look at previous blood results such as HbA1c – the average blood test you have approximately every 6 months. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

Your General Practitioner and the hospital Diabetes doctors will have been informed that the study is taking place. They will not be told of any findings in this study unless anything unusual is found in your blood and you have agreed for them to be told or they ask for a copy of the final results.

#### **What if I have a problem?**

If you have any complaints about staff or about something serious happening during or following your participation in the research please contact the researcher who will do her best to answer your questions:  
Kim Harman,





Lead Podiatrist diabetes,  
Podiatry clinic, Diabetes Centre,  
Royal United Hospital, Bath  
telephone number 01225 824061 direct line with an ansaphone when she is in.

Alternatively if you wish to complain formally you can do this through the NHS complaints procedure. The contact details for this process are:  
Patient Advice and Liaison Service (PALS) service,  
RUH Bath NHS trust,  
Combe Park, Bath BA1 3NG  
Telephone number 01225 825656, email [pals@ruh-bath.swest.nhs.uk](mailto:pals@ruh-bath.swest.nhs.uk),

#### **What if something goes wrong?**

In the event something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against an NHS trust but you may still have to pay your legal costs. Two different NHS trust are employing staff within this study. Additionally there is a mechanism for claiming from the insurance provided by the University. The normal National Health Service complaints mechanisms will still be available to you.

#### **Contact for further information about the study.**

For further information please contact Kim Harman, Lead Podiatrist diabetes, Podiatry, Diabetes Centre, Royal United Hospital, Bath. Telephone 01225 824061 direct line (with an ansaphone).

*Please note that I have now arranged with the hospital for you to be able to park for free when you attend for the scans providing I am given your original parking ticket in return.*

## 10.6 Consent form

Patient Identification Number for this trial:

## CONSENT FORM

**Can we improve how we diagnose osteomyelitis in the diabetic foot?**

Name of Researcher: Kim Haman

**Please initial the box at the end of the statement**

1. I confirm that I have read and understand the information sheet dated 21 December 2006 (version 2) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

☐

2. I am happy for my General Practitioner to be told I am taking part in this study.

☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.

☐

4. I understand that relevant sections of any of my medical notes and data collected during the study, may be looked at by responsible individuals from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.

☐

5. I agree to take part in the above study.

☐

6. I am happy / not happy to be told about any other health problem any blood test result may indicate and my General Practitioner / a medical consultant may be informed. (Delete as appropriate).

☐

Name of Patient

Date

Signature

Name of Person taking consent  
(if different from researcher)

Date

Signature

Researcher

Date

Signature

When completed, 1 for patient; 1 for researcher site file; 1 (original) to be kept in medical notes

## 10.7 Data collection tool

Data collection tool research v6

Name

DoB

Hospital number

Consented date

Date of assessment

S(AD) SAD score

Grade	Area	Depth	Sepsis	Ateriopathy	Denervation
0	Skin intact	Skin intact	No infection	Pedal pulses palpable	Pinprick sensation/VPT normal
1	< 10 mm <sup>2</sup>	Skin and subcutaneous tissues	Superficial slough or exudate	Diminution of both pulses or absence of one	Reduced or absent pinprick sensation or VPT raised
2	10-30 mm <sup>2</sup>	Tendon, joint capsule	Cellulitis	Absence of both pedal pulses	Neuropathy dominant palpable pedal pulses
3	> 30 mm <sup>2</sup>	Bone and/or joint spaces	Osteomyelitis	Gangrene	Charcot foot

Total ulcer score

PEDIS assessment

Perfusion

Grade 1 (no signs/symptoms PAD)

Grade 2 (signs/symptoms of PAD but not critical ischaemia)

Grade 3 (critical limb ischaemia)

Extent/size

mm<sup>2</sup>

Depth/tissue loss

Grade 1 (superficial dermis only)

Grade 2 (deep to fascia, muscle or tendon)

Grade 3 (bone and/or joint)

Infection

Grade 1 (no signs/symptoms)

Grade 2 (skin and subcutaneous tissue, erythema 0.5-2 cm)

Grade 3 (erythema >2cm plus tenderness/warmth/purulent discharge)

Grade 4 (systemic inflammatory response)

Sensation

Grade 1 (no loss)

Grade 2 (loss of 2 of 3 10g monofilament test sites / absent vibration)

Bloods taken Yes/no/given form (delete) Date

Possible changes in bloods due to

Blood results obtained

Hb	Neutrophil count
PV	CRP
PCN	

Referred to MRI Yes/no (delete) Date

Referred to SPECT/CT Yes/no (delete) Date

Date of MRI

Date of SPECT/CT

Date of 2<sup>nd</sup> blood taken yes/no/form given (delete)

Blood results obtained

Hb	Neutrophil count
PV	CRP
PCN	

Age

Type of diabetes

Duration of disease

Use of Metformin YES / NO

Use of insulin YES / NO

Use of other OHA YES / NO

History of CVD YES / NO

History of PVD YES / NO

ID No for this trial

Visit number

## 10.8 Proposed consensus criteria for diagnosing osteomyelitis in the diabetic foot

From Berendt *et al* (2008)

Table 1. Proposed consensus criteria for diagnosing osteomyelitis in the diabetic foot

Category	Post-test probability of osteomyelitis	Management advice	Criteria	Comments
Definite ('beyond reasonable doubt')	>90%	Treat for osteomyelitis	Bone sample with positive culture AND positive histology OR Purulence in bone found at surgery OR Atraumatically detached bone fragment removed from ulcer by podiatrist/surgeon OR Intraosseous abscess found on MRI OR	Sample must be obtained at surgery or through uninvolved skin Definite purulence identified by experienced surgeon Definite bone fragment identified by experienced surgeon/podiatrist
Probable ('more likely than not')	51–90%	Consider treating, but further investigation may be needed	Any two probable criteria OR one probable and two possible criteria OR any four possible criteria below Visible cancellous bone in ulcer OR MRI showing bone oedema with other signs of osteomyelitis OR Bone sample with positive culture but negative or absent histology OR Bone sample with positive histology but negative or absent culture OR	Sinus tract, sequestrum, heel or metatarsal head involved; cloaca
Possible (but on balance, less rather than more likely)	10–50%	Treatment may be justifiable, but further investigation usually advised	Any two possible criteria below Plain X-rays show cortical destruction OR MRI shows bone oedema OR cloaca OR Probe to bone positive OR, Viable cortical bone OR ESR > 70 mm/h with no other plausible explanation OR	
Unlikely	<10%	Usually no need for further investigation or treatment	Non-healing wound despite adequate offloading and perfusion for >6 weeks OR ulcer of >2 weeks duration with clinical evidence of infection No signs or symptoms of inflammation AND normal X-rays AND ulcer present for >2 weeks or absent AND any ulcer present is superficial OR Normal MRI OR Normal bone scan	



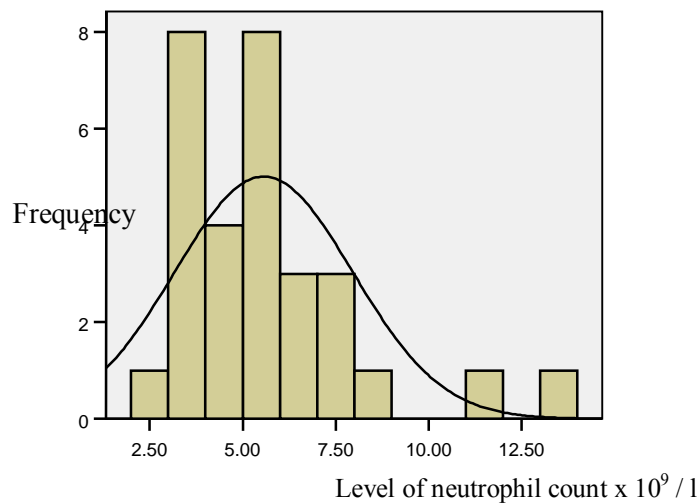
## 10.9 Histograms and summary statistics of each inflammatory marker in each wound condition

### Neutrophil Count in clean wounds

Normal range is  $2 - 7.5 \times 10^9 / l$

Descriptive Statistics	Minimum	Maximum	Mean	Std. Deviation
Neutrophil Count	2.50	13.30	5.57	2.39

Histogram of Neutrophil Count in clean wounds with normal curve

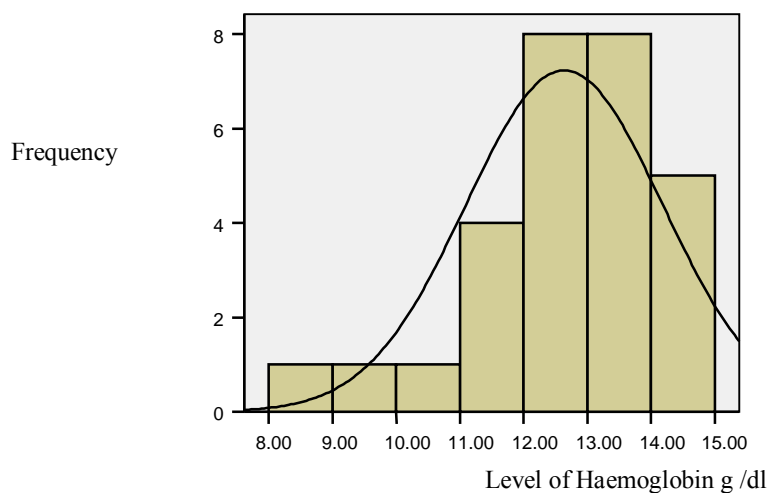


### Haemoglobin in clean wounds (not including renal impairment)

Normal range is ♀  $11.5 - 15.5 \text{ g / dl}$  ♂  $13.5 - 15.5 \text{ g / dl}$  anaemia is  $< 11 \text{ g / dl}$

Descriptive Statistics	Minimum	Maximum	Mean	Std. Deviation
Haemoglobin	8.60	14.80	12.64	1.54

Histogram of Haemoglobin in clean wounds with normal curve

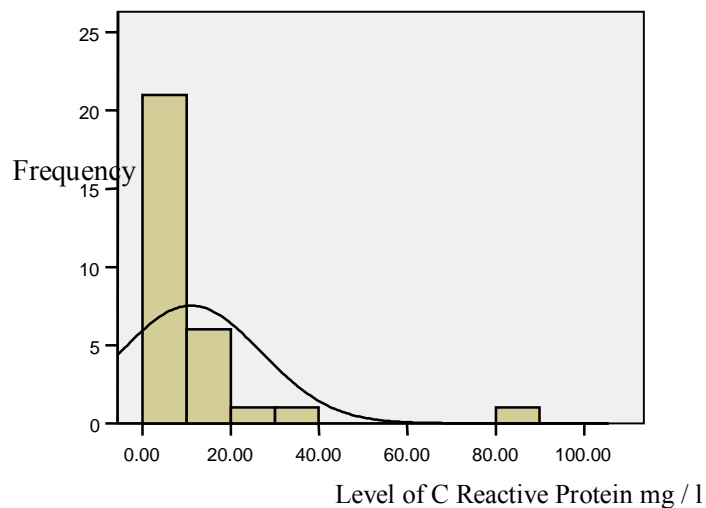


### CRP in clean wounds

Normal is less than 5mg/l

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation	Median
C Reactive Protein	4.99	89.00	11.03	15.89	11.08

Histogram of C Reactive Protein in clean wounds with normal curve

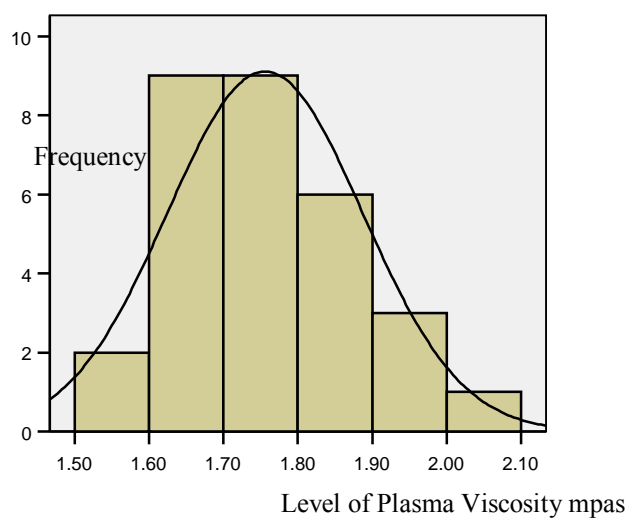


### Plasma Viscosity in clean wounds

Normal range is 1.5 – 1.72 mpas

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Plasma Viscosity	1.50	2.10	1.76	0.13

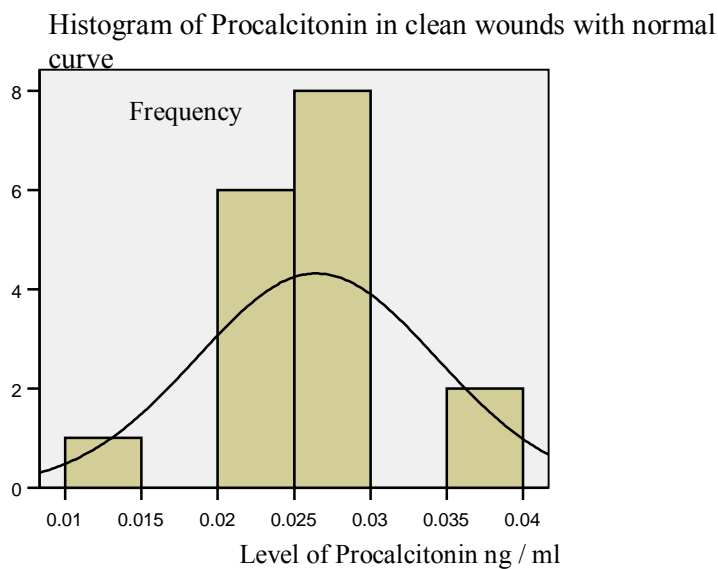
Histogram of Plasma Viscosity in clean wounds with normal curve



### Procalcitonin in clean wounds

Normal range in 'healthy' individuals <0.3ng/ml

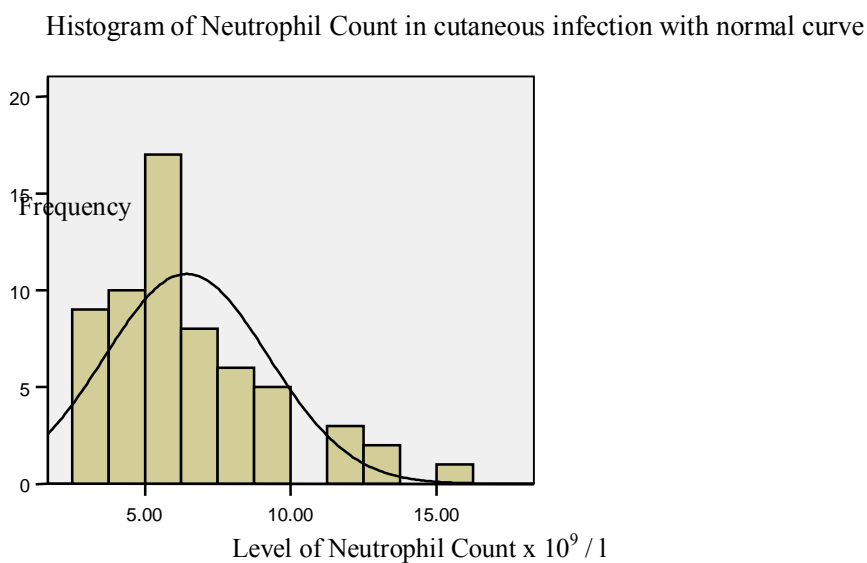
<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation	Median
Procalcitonin	0.01	0.04	0.03	0.00	0.3



### Neutrophil Count in cutaneous infection

Normal range is  $2 - 7.5 \times 10^9 / l$

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Neutrophil Count	2.80	16.10	6.43	2.81

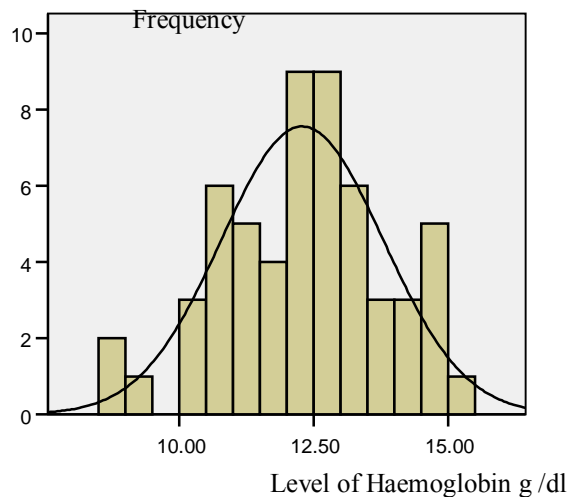


### Haemoglobin in cutaneous infection (not including renal impairment)

Normal range is ♀ 11.5 – 15.5 g /dl ♂ 13.5 – 15.5 g / dl anaemia is < 11g /dl

Descriptive Statistics	Minimum	Maximum	Mean	Std. Deviation
Haemoglobin	8.60	15.10	12.29	1.50

Histogram of Haemoglobin in cutaneous infection with normal curve

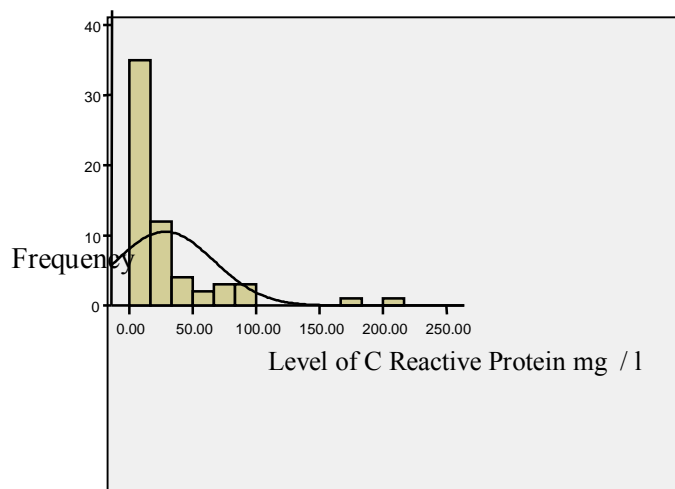


### CRP in cutaneous infection

Normal range is less than 5mg/l

Descriptive Statistics	Minimum	Maximum	Mean	Std. Deviation	Median
C Reactive Protein	4.99	215.00	28.16	38.51	13.00

Histogram of C Reactive Protein in cutaneous infection with normal curve

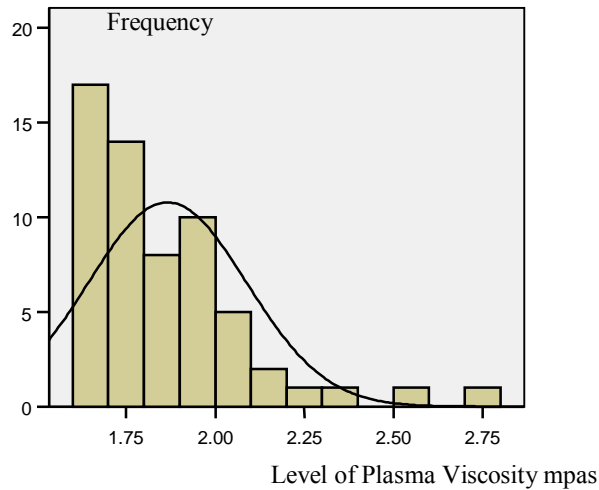


### Plasma Viscosity in cutaneous infection

Normal range is 1.5 – 1.72 mpas

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Plasma Viscosity	1.62	2.72	1.87	0.22

Histogram of Plasma Viscosity in cutaneous infection with normal curve

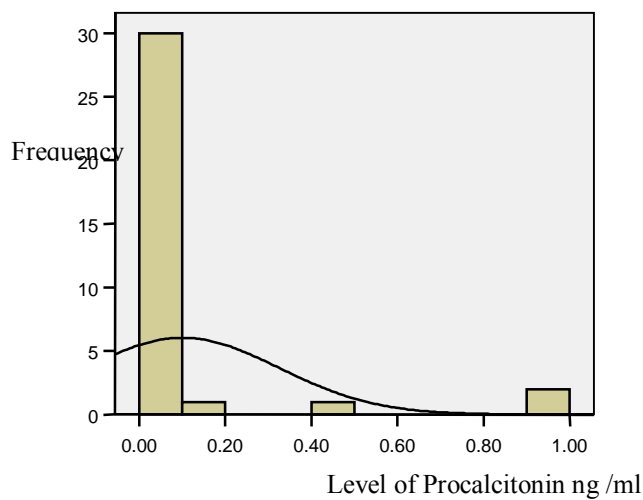


### Procalcitonin in cutaneous infection

Normal range in 'healthy' individuals <0.3ng/ml

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation	Median
Procalcitonin	0.01	0.93	0.10	0.22	0.03

Histogram of Procalcitonin in cutaneous infection with normal curve

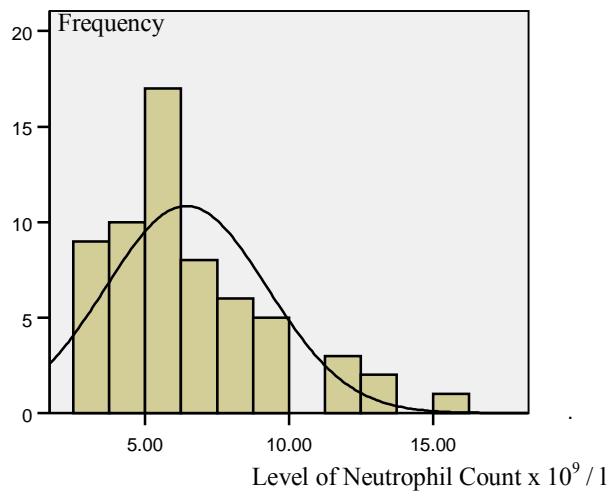


### Neutrophil Count in suspected osteomyelitis

Normal range is  $2 - 7.5 \times 10^9 / l$

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Neutrophil Count	2.80	16.10	6.34	2.94

Histogram of Neutrophil Count in suspected osteomyelitis with normal curve

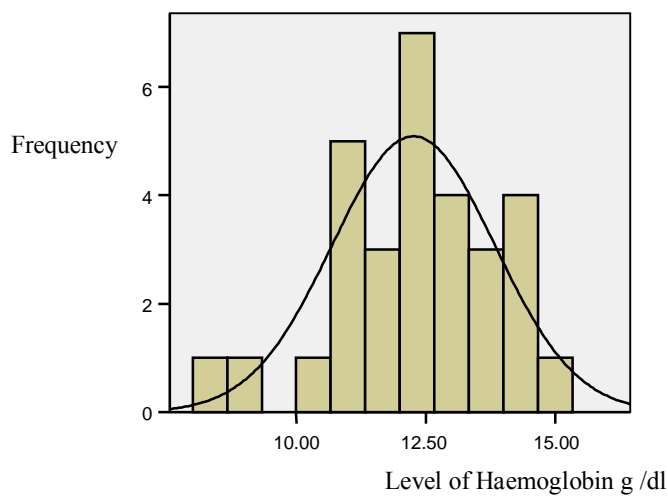


impairment)

Normal range is ♀ 11.5 – 15.5 g /dl ♂ 13.5 – 15.5 g / dl anaemia is < 11 g /dl

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Haemoglobin	8.60	15.10	12.26	1.57

Histogram of Haemoglobin in suspected osteomyelitis with normal curve

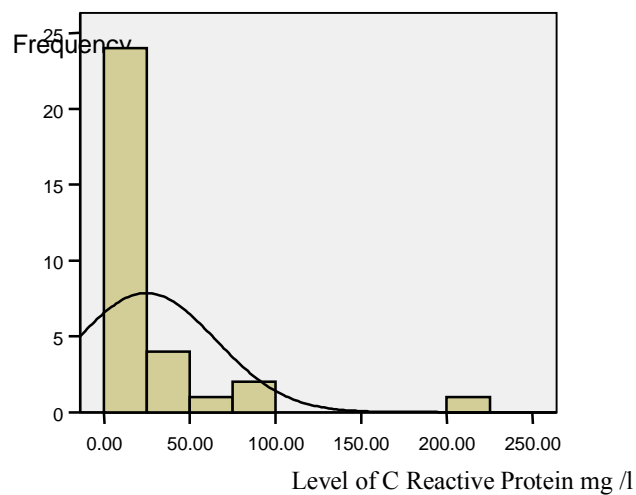


### CRP in suspected osteomyelitis

Normal range is below 5mg/l

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation	Median
C Reactive Protein	4.99	215.00	24.53	40.65	10.50

Histogram of C Reactive Protein in suspected osteomyelitis with normal curve

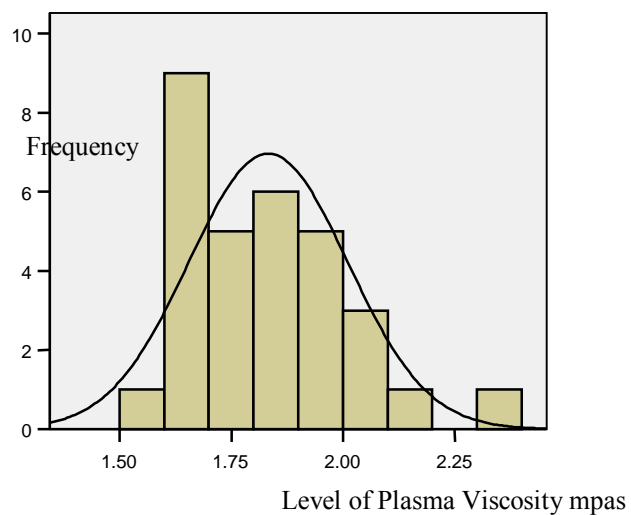


### Plasma Viscosity in suspected osteomyelitis

Normal range is 1.5 – 1.72 mpas

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Plasma Viscosity	1.53	2.38	1.83	0.18

Histogram of Plasma Viscosity in suspected osteomyelitis with normal curve



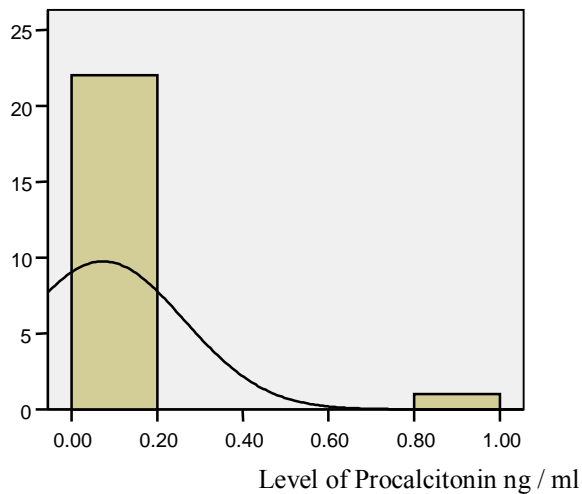


### Procalcitonin in suspected osteomyelitis

Normal range in 'healthy' individuals <0.3ng/ml

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation	Median
Procalcitonin	0.02	0.93	0.07	0.19	0.03

Frequency  
Histogram of Procalcitonin in suspected osteomyelitis with normal curve

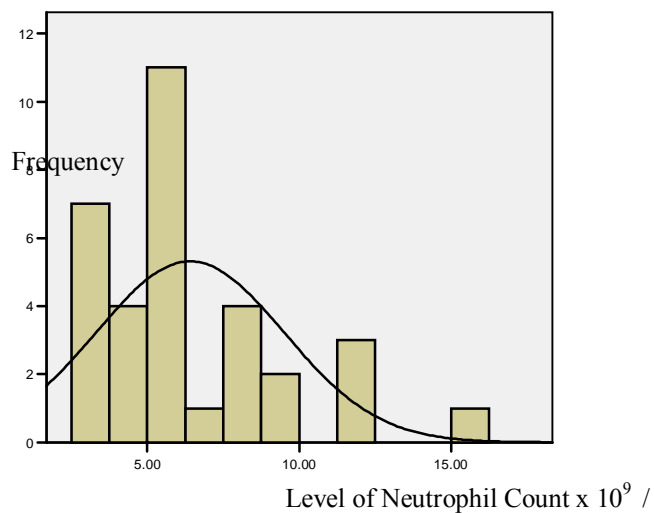


### Neutrophil Count in proven osteomyelitis

Normal range is  $2 - 7.5 \times 10^9 / l$

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Neutrophil Count	2.80	16.10	6.41	3.10

Neutrophil Count in confirmed osteomyelitis with normal curve

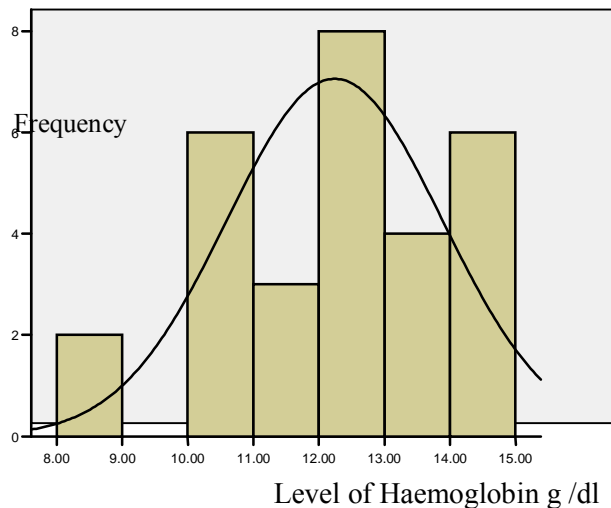


### Haemoglobin in proven osteomyelitis (not including renal impairment)

Normal range is ♀ 11.5 – 15.5 g /dl ♂ 13.5 – 15.5 g / dl anaemia is < 11 g /dl

Descriptive Statistics	N	Minimum	Maximum	Mean	Std. Deviation
Haemoglobin	29	8.60	14.70	12.24	1.64

Histogram of Haemoglobin in confirmed osteomyelitis with normal curve

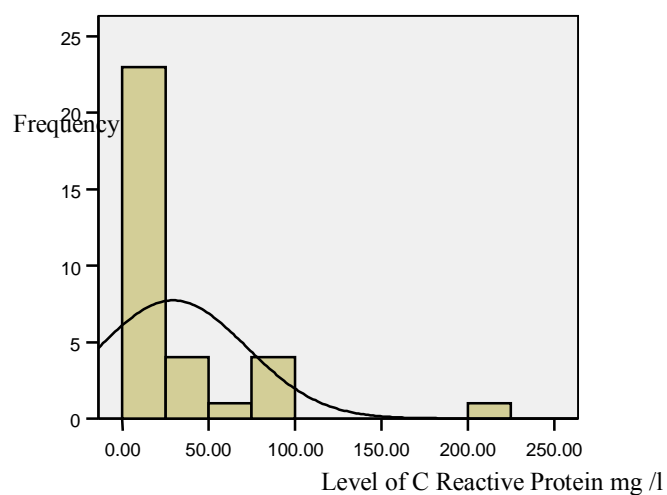


### CRP in proven osteomyelitis

Normal range is less than 5mg/l

Descriptive Statistics	Minimum	Maximum	Mean	Std. Deviation
C Reactive Protein	4.99	215.00	29.33	42.58

Histogram of C Reactive Protein in confirmed osteomyelitis with normal curve

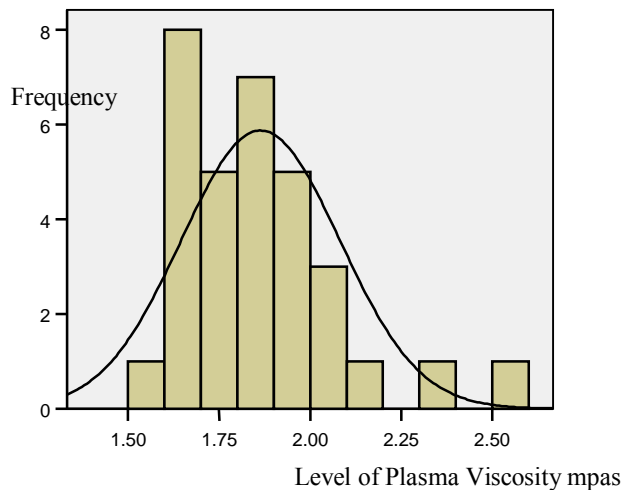


### Plasma Viscosity in proven osteomyelitis

Normal range is 1.5 – 1.72 mpas

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation
Plasma Viscosity	1.53	2.59	1.86	0.22

Histogram of Plasma Viscosity in confirmed osteomyelitis with normal curve

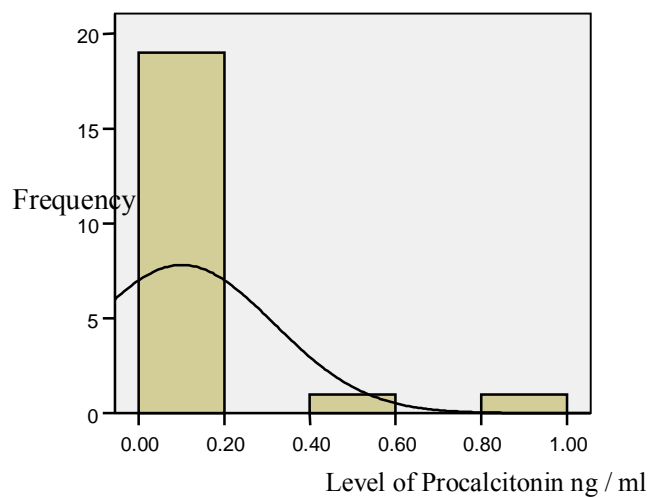


### Procalcitonin in proven osteomyelitis

Normal range in 'healthy' individuals <0.3ng/ml

<b>Descriptive Statistics</b>	Minimum	Maximum	Mean	Std. Deviation	Median
Procalcitonin	0.02	0.93	0.10	0.21	0.30

Histogram of Procalcitonin in confirmed osteomyelitis with normal curve



## 10.10 Regression analysis results

### 10.10.1 Regression models without confounders

#### 10.10.1.1 Regression models without confounders in clean wounds

##### Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
								Lower	Upper
Step 1	PV	4.344	2.243	3.752	1	.053	77.018	.950	6245.649
1(a)	Constant	-7.396	4.058	3.322	1	.068	.001		

a Variable(s) entered on step 1: PV.

##### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 PV	-30.846	5.911	1	.015

a Based on conditional parameter estimates

##### Variables not in the Equation

			Score	df	Sig.
Step 1	Variables	Ncount	.709	1	.400
		Hb	.273	1	.601
		CRP	2.354	1	.125
		PCN	1.122	1	.289
Overall Statistics			3.809	4	.432

#### 10.10.1.2 Regression models without confounders in cutaneous infected wounds

##### Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
								Lower	Upper
Step 1	Ncount	-.362	.181	4.003	1	.045	.696	.488	.993
1(a)	Constant	1.185	1.014	1.364	1	.243	3.270		

a Variable(s) entered on step 1: Ncount.

#### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 Ncount	-28.707	5.325	1	.021

a Based on conditional parameter estimates

#### Variables not in the Equation

	Score	df	Sig.
Step 1 Variables Hb	.515	1	.473
CRP	1.684	1	.194
PV	1.615	1	.204
PCN	.853	1	.356
Overall Statistics	2.779	4	.595

#### 10.10.1.3 Regression models without confounders in suspected osteomyelitis

#### Variables not in the Equation

	Score	df	Sig.
Step 0 Variables Ncount	.094	1	.759
Hb	.353	1	.552
CRP	.416	1	.519
PV	.228	1	.633
PCN	.270	1	.604
Overall Statistics	1.620	5	.899

#### 10.10.1.4 Regression models without confounders in confirmed osteomyelitis

#### Variables not in the Equation

	Score	df	Sig.
Step 0 Variables Ncount	.125	1	.724
Hb	.218	1	.641
CRP	.088	1	.766
PV	.203	1	.652
PCN	2.657	1	.103
Overall Statistics	2.961	5	.706

## 10.10.2 Regression models with confounders

### 10.10.2.1 Regression models with confounders in clean wounds

#### confounder not on antibiotics

##### Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
								Lower	Upper
Step 1(a)	CRP	.147	.082	3.193	1	.074	1.158	.986	1.361
	Constant	-2.106	1.098	3.680	1	.055	.122		

a Variable(s) entered on step 1: CRP.

##### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 CRP	-12.020	7.738	1	.005

a Based on conditional parameter estimates

##### Variables not in the Equation

			Score	df	Sig.
Step 1	Variables	Ncount	1.543	1	.214
		Hb	.104	1	.747
		PV	.206	1	.650
		PCN	.558	1	.455
Overall Statistics			1.933	4	.748

#### confounder on Insulin

##### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	3.749	1	.053
		Hb	.171	1	.679
		CRP	2.489	1	.115
		PV	3.116	1	.078
		PCN	1.113	1	.291
Overall Statistics			4.599	5	.467

### confounder on Metformin

#### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	1.678	1	.195
		Hb	2.005	1	.157
		CRP	2.042	1	.153
		PV	2.026	1	.155
		PCN	.946	1	.331
	Overall Statistics		4.042	5	.543

### confounder predominant etiology neuropathy

#### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	1.773	1	.183
		Hb	1.574	1	.210
		CRP	3.761	1	.052
		PV	3.265	1	.071
		PCN	.968	1	.325
	Overall Statistics		5.706	5	.336

### 10.10.2.2 Regression models with confounders in cutaneous infected wounds

### confounder not on antibiotics

#### Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
								Lower	Upper
Step 1(a)	Ncount	-.608	.364	2.789	1	.095	.544	.267	1.111
	Constant	3.774	2.094	3.249	1	.071	43.554		

a Variable(s) entered on step 1: Ncount.

#### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 Ncount	-11.523	4.700	1	.030

a Based on conditional parameter estimates

#### Variables not in the Equation

	Score	df	Sig.
Step 1 Variables Hb	.176	1	.675
CRP	1.406	1	.236
PV	1.687	1	.194
PCN	.075	1	.784
Overall Statistics	2.312	4	.679

#### confounder on Insulin

#### Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
							Lower	Upper
Step 1(a) Ncount	-.582	.277	4.434	1	.035	.559	.325	.961
Constant	2.409	1.477	2.661	1	.103	11.126		

a Variable(s) entered on step 1: Ncount.

#### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 Ncount	-16.285	7.646	1	.006

a Based on conditional parameter estimates

#### Variables not in the Equation

	Score	df	Sig.
Step 1 Variables Hb	.030	1	.862
CRP	.037	1	.848
PV	.087	1	.768
PCN	.068	1	.794
Overall Statistics	.316	4	.989



## confounder on Metformin

### Variables not in the Equation

		Score	df	Sig.
Step 0	Variables			
	Ncount	.643	1	.423
	Hb	2.436	1	.119
	CRP	1.627	1	.202
	PV	.971	1	.324
	PCN	.777	1	.378
Overall Statistics		3.073	5	.689

## confounder predominant etiology neuropathy

### Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
								Lower	Upper
Step 1(a)	Ncount	-.439	.222	3.907	1	.048	.645	.417	.996
	Constant	1.615	1.194	1.831	1	.176	5.029		

a Variable(s) entered on step 1: Ncount.

### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 Ncount	-23.362	4.852	1	.028

a Based on conditional parameter estimates

### Variables not in the Equation

		Score	df	Sig.
Step 1	Variables			
	Hb	.369	1	.543
	CRP	1.043	1	.307
	PV	.377	1	.539
	PCN	.819	1	.366
Overall Statistics		1.626	4	.804

### 10.10.2.3 Regression models with confounders in suspected osteomyelitis

#### confounder not on antibiotics

##### Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
								Lower	Upper
Step 1(a)	PV	-13.051	6.927	3.550	1	.060	.000	.000	1.691
	Constant	25.071	12.835	3.816	1	.051	77329155 073.191		

a Variable(s) entered on step 1: PV.

##### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 PV	-10.303	7.890	1	.005

a Based on conditional parameter estimates

##### Variables not in the Equation

			Score	df	Sig.
Step 1	Variables	Ncount	.206	1	.650
		Hb	.209	1	.647
		CRP	.208	1	.649
		PCN	.198	1	.656
		Overall Statistics	2.578	4	.631

#### confounder on Insulin

##### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	.103	1	.748
		Hb	1.597	1	.206
		CRP	.242	1	.623
		PV	.047	1	.828
		PCN	.842	1	.359
		Overall Statistics	3.628	5	.604

### confounder on Metformin

#### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	.176	1	.674
		Hb	.019	1	.891
		CRP	.165	1	.685
		PV	.135	1	.713
		PCN	1.259	1	.262
	Overall Statistics		1.627	5	.898

### confounder predominant etiology neuropathy

#### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	.814	1	.367
		Hb	.222	1	.638
		CRP	.165	1	.685
		PV	.006	1	.938
		PCN	.896	1	.344
	Overall Statistics		3.279	5	.657

### 10.10.2.4 Regression models with confounders in confirmed osteomyelitis

### confounder not on antibiotics

#### Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I. for EXP(B)	
								Lower	Upper
Step 1(a)	PV	-7.482	4.537	2.719	1	.099	.001	.000	4.101
	Constant	15.180	8.536	3.162	1	.075	3911964.001		

a Variable(s) entered on step 1: PV.

#### Model if Term Removed(a)

Variable	Model Log Likelihood	Change in - 2 Log Likelihood	df	Sig. of the Change
Step 1 PV	-9.277	4.393	1	.036

a Based on conditional parameter estimates

#### Variables not in the Equation

			Score	df	Sig.
Step 1	Variables	Ncount	.053	1	.818
		Hb	.202	1	.653
		CRP	.150	1	.698
		PCN	.127	1	.722
		Overall Statistics	1.724	4	.786

#### confounder on Insulin

#### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	1.337	1	.248
		Hb	.404	1	.525
		CRP	2.606	1	.106
		PV	.620	1	.431
		PCN	1.079	1	.299
		Overall Statistics	3.296	5	.654

#### confounder on Metformin

#### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Ncount	.236	1	.627
		Hb	.144	1	.704
		CRP	.099	1	.753
		PV	.264	1	.607
		PCN	1.886	1	.170
		Overall Statistics	2.208	5	.820

confounder predominant etiology neuropathy

**Variables not in the Equation**

			Score	df	Sig.
Step 0	Variables	Ncount	.361	1	.548
		Hb	.681	1	.409
		CRP	.020	1	.887
		PV	.001	1	.981
		PCN	1.394	1	.238
	Overall Statistics		2.943	5	.709

## **References**

Adetunji, O.R., Mani, H., Morgan, C. and Gill, G.V. 2009. Metformin and anaemia: myth or reality? *Practical Diabetes International*, 26(7), 265 – 266.

Ahmadi, M.R., Morrison, W.B., Carrino, J.A., Schweitzer, M.E., Raikin, S.M. and Ledermann, H.P. 2006. Neuropathic Arthropathy of the Foot with and without Superimposed Osteomyelitis: MR Imaging Characteristics. *Radiology*, 238(2), 622 – 631.

Al-Gwaiz, L. and Babay, H. 2007. The Diagnostic value of Absolute Neutrophil Count, Band Count and Morphologic Changes of Neutrophils in Predicting Bacterial Infections. *Medical Principles and Practice*. 16, 344 - 347.

Al-Khoury, S., Afzali, B., Shah, N, Covie, A, Thomas, S. and Goldsmith, D. 2006. Anaemia in diabetic patients with chronic kidney disease – prevalence and predictors. *Diabetologia*. 49, 1183 - 1189.

American Diabetes Association and the American Academy of Neurology, 1988. *Report and Recommendations of the San Antonio Conference on Diabetic Neuropathy, 8-10 February, 1988*. San Antonio Texas. *Diabetes*. 37(7), 1000 - 1004.

Amos, A.F., McGarty, D.J., and Zimmet, P., 1997. The Rising Global Burden of Diabetes and its complications: Estimates to the year 2010. *Diabetic Medicine*, Supplement 14, S7 - S85.

Apelqvist, J. Ragnarsson -Tenvall, G, and Persson, U., 1994. Diabetic foot ulcers in a multidisciplinary setting – an economic analysis of primary healing and healing with amputation. *Journal of Internal Medicine*, 235, 463 - 471.

Apelqvist, J., Bakker, K., van Houtum, W., Nabuurs-Franssen, M and Schaper, N. 1999. International Working Group on the Diabetic Foot. Maastricht.

Armstrong, D.G., Lavery, L.A. and Harkless, L.B., 1998. Validation of a Diabetic Wound Classification, the contribution of depth, infection, and ischemia to risk of amputation. *Diabetes Care*, 21(5), 855 - 859.

Armstrong, D.G., Lavery, L.A, Sariaya, M. and Ashry, H., 1996a. Leukocytosis is a poor indicator of acute osteomyelitis of the foot in diabetes mellitus. *Journal of Foot and Ankle Surgery*, 35(280), 280 - 283.

Armstrong, D.G., Liswood, P.L. and Todd, W.F. 1995. Prevalence of mixed infections of the diabetic pedal wound. *Journal of the American Podiatric Medical Association* 85(10), 533 – 537.

Armstrong, D.G., Perales, T.A., Murff, R.T., Edelson, G.W. and Welchon, J.G. 1996b. Value of White Blood Cell Count With Differential in the Acute Diabetic Foot Infection. *Journal of the American Podiatric Medical Association*. 86(5), 224 - 227.

Bagdale, J, Root, R, and Bulger, R., 1974. Impaired leukocyte function in patients with poorly controlled diabetes. *Diabetes*, 23, 9 - 14.

Bakker, K., Foster, A., van Houtum, W., Riley, P. eds., 2005. *Put feet first to prevent amputations Diabetes and Footcare Time to Act*. Brussels: International Diabetes Federation and the International Working Group on the Diabetic Foot.

Bamberger, D.M., Daus, G.P. and Gerding, D.N., 1987. Osteomyelitis in the feet of diabetic patients: long-term results, prognostic factors, and the role of antimicrobial and surgical therapy. *American Journal of Medicine*, 83(4), 653-660.

Balshaw-Greer, A., Davies, J. and Casey, J., 2005. Is anaemia in patients with diabetes exclusive to diabetic nephropathy? *Journal of Diabetes Nursing*, 9(1), 24 - 27.

Becker, K., Snider, R. and Nylen, E. 2008. Procalcitonin assay in systemic inflammation, infection, and sepsis: Clinical utility and limitations. *Critical Care Medicine*. 36(3), 941 - 952.

Bell, K., Wattie, M., Silverstrini, R., Clark, P., Stachowski, E. and Benson, E. 2003. Procalcitonin: A marker of Bacteraemia in SIRS, *Anaesthesia and Intensive Care*, 31(6), 629-636.

Benotmane, A., Mohammedi, F., Ayad, F., Kadi, K. and Azzouz, A., 2000. Diabetic Foot Lesions. *Diabetes and Metabolism*, 26(2,) 113 - 117.

Berendt, A. and Lipsky, B., 2004. Is This Bone Infected or Not? Differentiating Neuro-osteoarthropathy from Osteomyelitis in the Diabetic Foot. *Current Diabetes Reports*, 4(6), 424 - 429.

Berendt, A., Peters, E., Bakker, K., Embil, J., Eneroth, M., Hinchcliffe, R., Jeffcoate, W., Lipsky, B., Senneville, E., Teh, J. and Valk, G.. 2008. Diabetic foot osteomyelitis: a progress report on diagnosis and a systematic review of treatment. *Diabetes Metabolism Research and Reviews*. 24 (suppl 1) S 145 -S161.

Boorgula, V., Flanagan, J., Conry, G. and Fordyce, M.J., 2004. Further Evaluation of Diagnostic accuracy of LeukoScan in suspected Osteomyelitis and prosthetic Infection. *Nuclear Medicine Communications*, 25(4), 404.

Bosman, D., Winkler, A., Marsden, J., Macdougall, I.C. and Watkins, P.J., 2001. Anemia With Erythropoietin Deficiency Occurs Early in Diabetic Nephropathy. *Diabetes Care*, 24(3), 495 - 499.



Boulton, A.J.M., Vileikye, L., Ragnarson-Tenvall, G. and Apelqvist, J., 2005. The global burden of diabetic foot disease. *The Lancet*, 366: 1719 - 1724.

Boutin, R.D., Brossman, J., and Sartoris, D. 1998. Update on imaging of orthopaedic infection. *Orthopedic Clinics Of North America*, 29, 41-66. In: Paluska, S., 2004. Osteomyelitis. *Clinics in Family Practice*. 6(1), 127 - 156.

Boyko, E., Ahroni, J., Smith, G. and Davignon, D., 1996. Increased mortality associated with diabetic foot ulcer. *Diabetic Medicine*, 13(11), 770 - 776.

BRAHMS PCT LIA version 05us. Available at: <URL<http://www.brahms-usa.com/manuals/PCT%20LIA520u5us.pdf>> [Accessed 23 October 2009]

Bridgen, M. 1999. Clinical Utility of the Erythrocyte Sedimentation Rate. *American Family Physician*, 60(5), 1443 - 1455.

Burton, K. 2001. Peripheral neurological testing procedures in screening. *The Diabetic Foot*, 4(2), 87 - 92.

Butbul-Aviel, Y., Koren, A., Halvey, R., and Sakran, W., 2005. Procalcitonin as a Diagnostic aid in Osteomyelitis and Septic Arthritis. *Pediatric Emergency Care*, 21(12), 828 - 832.

Boulton, A., Vileikye, L., Ragnarson-Tenvall, G. and Apelqvist, J., 2005. The global burden of diabetic foot disease. *The Lancet*, 366, 1719 - 1724.

Carragee, E.J., Kim, D., van der Vlugt, T. and Vittum, D., 1997. The Clinical Use of Erythrocyte Sedimentation Rate in Pyogenic Vertebral Osteomyelitis. *Spine*, 22(18), 2089 - 2093.

Carter, A., Bennett, C., Bostock, J. and Grant, P.J., 2005. Metformin reduces C-reactive protein but not complement factor C3 in overweight patients with Type 2 diabetes mellitus. *Diabetic Medicine*, 22(9), 1282 - 1284.

Casella, J.P., Ashcroft, R.L. and Kavanagh-Sharp, V., 2000. Effect of applied pressure in the determination of vibration sensitivity using the Neurothesiometer. *The Foot*, 10, 27 - 30.

Castelli, G., Pognani, C., Meisner, M., Stuani, A., Bellomi, D., and Sgarbi, L. 2004. Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. *Critical care*. 8(4), R234 -242.

Cavanagh, P., Lipsky, B., Bradbury, A. and Botek, G., 2005. Treatment for diabetic foot ulcers. *The Lancet*, 366, 1725 - 1735.

Chatha, D.S., Cunningham, P.M. and Schweitzer, M.E., 2005. MR Imaging of the Diabetic Foot: Diagnostic Challenges. *Radiologic Clinics of North America*, 43, 747 - 759.

Chiesa, C., Panero, A. and Rossi, N., 1998. Reliability of Procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. *Clinical Infectious Diseases*, 26, 664 - 672.

Chu, N., Kong, A., Kim, D., Armstrong, D., Baxi, S., Deutsch, R., Caulfield, M., Mudaliar, S., Reitz, R., Henry, R. and Reaven, P., 2002. Differential effects of Metformin and Troglitazone on cardiovascular risk Factors in Patients With Type 2 Diabetes. *Diabetes Care*, 25(3), 542 - 549.

Chudleigh, R., Dunseath, G., Peter, R., Harvey, j., Ollerton, R., Luzio, S. and Owens, D. 2008. Influence of Body weight on the Performance of Glomerular Filtration rate Estimators in Subjects With Type 2 Diabetes, *Diabetes Care*, 31(1), 47 - 49.

Cogdon, P., 2006. Estimating diabetes prevalence by small area in England. *Journal of Public Health* (Oxford University Press), 28, 71 – 81.

Coppola, L., Verrazzo, G., La Marca, C., Ziccardi, A., Grassia, P., Tirelli, D, and Giugliano, D., 1997. Effect of Insulin on Blood Rheology in Non-diabetic Subjects and in Patients with Type 2 Diabetes Mellitus. *Diabetic Medicine*, 14, 959 - 963.

Covey, D., and Albright, J., 1987. Clinical Significance of the Erythrocyte Sedimentation Rate in Orthopaedic Surgery. *The Journal of Bone and Joint Surgery (Am)*, 69A(1), 148 - 151.

Craig, J.G., Amin, M.B., Wu, K., Eyler, W., van Holsbeeck, M., Bouffard, J. and Shirazi, K., 1997. Osteomyelitis of the diabetic foot; MR imaging-pathologic correlation. *Radiology*, 203(3), 849 - 855.

Craig, K., Williams, J., Riley, S., Smith, H., Owends, D., Worthing, D., Cavill, I. and Phillips, A., 2005. Anemia and Diabetes in the Absence of Nephropathy. *Diabetes Care*, 28(5), 1118 - 1123.

Crim, J.R. and Seegar, L.L., 1994. Imaging evaluation of osteomyelitis. *Critical Reviews in Diagnostic Imaging*, 35(3), 201 - 256.

Crolle, S.D., Nicholas, G.G., Osborne, M.A., Wasser, T. and Jones, S., 1996. Role of magnetic resonance imaging in the diagnosis of osteomyelitis in diabetic foot infections. *Journal of Vascular Surgery*, 24(2), 266 - 270.

Cunha, B.A., 2002. Osteomyelitis in Elderly Patients. *Aging and Infectious Diseases*, 35, 287 - 293.

Currie, C.J., Williams, D.R., and Peters, J.R., 1996. Patterns of in and out- patient activity for diabetes: a district survey. *Diabetes Medicine*, 13, 273 - 280.

Davey-Smith, G., Timpson, N and Lawlor, D., 2006. C-Reactive Protein and cardiovascular Disease Risk: Still an Unknown Quantity? *Annals of Internal Medicine*, 145(1), 70 - 72.

Delcourt, A., Huglo, D., Prangere, T., Benticha, H., Devemy, F., Tsirtsikoulou, D., Lepeut, M., Fontaine, P. and Steinling, M., 2005. Comparison between Leukoscan® (Sulesomab) and Gallium-67 for the diagnosis of osteomyelitis in the diabetic foot. *Diabetes Metabolism*, 31, 125 - 133.

Delévaux, I., André, M., Colombier, M., Albuisson, E., Meylheuc, F., Bégue, R-J., Piette, J-C and Aumaître, O. 2003. Can Procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? *Annals of Rheumatic Disease*. 62, 37-340.

Diabetes UK 2006. *New figures predict worrying rise in diabetes* [on line] London. Available at: < [URL:http://www.diabetes.org.uk/About\\_us/News-Landing-page/2573/](http://www.diabetes.org.uk/About_us/News-Landing-page/2573/) >[Accessed 16 October 2006].

Dinh, M.T., Abad, C.L. and Safdar, N. 2008. Diagnostic Accuracy of the Physical examination and Imaging Tests for Osteomyelitis Underlying Diabetic Foot Ulcers: Meta-analysis. *Clinical Infectious Diseases*. 47(15), 519 – 527.

Eberhard, O., Haubitz, M., Brunkhorst, F., Kleim, V., Koch, K. and Brunkhorst, R. 1997. Usefulness of Procalcitonin for differentiation between activity of systemic autoimmune disease (systemic lupus erythematosus/systemic antineutrophil cytoplasmic antibody-associated vasculitis) and invasive bacterial infection. *Arthritis and Rheumatism*. 40(7), 1250 - 1256.

Ebskov, L.B., Schroeder, P.E. and Holstein, P.E., 1994. Epidemiology of leg amputation: the influence of vascular surgery. *The British Journal of Surgery*. 81, 1600 – 1603.

Eckman, M.H., Greenfield, S., Mackey, W.C., Wong, J.B., Kaplan, S., Sullivan, L., Dukes, K. and Pauker, S.G., 1995. Foot infections in diabetic patients. Decisions and cost-effectiveness analyses. *Journal of the American Podiatric Association*, 273, 712 - 720.

Edmonds, M.E., Blundell, M.P., Morris, M.E., Maelor Thomas, E., Cotton, L.T., and Watkins, P.J., 1986. Improved survival of the Diabetic Foot: The Role of a Specialised Foot Clinic. *Quarterly Journal of Medicine*, 60(232), 763 - 771.

Elkeles, R.S and Wolfe, J.H., 1991. The diabetic foot. *British Medical Journal*, 303 1053 - 1055.

El-Shazly, M., Abdel-Fattah, M., Scorpiglione, N. Benedetti, M.M., Capani, F., Carinci, F., Carta, Q., Cavaliere, D., De Feo, E.M., Taboga, C., Tognoni, G. and Nicolucci, A., 1998. Risk factors for lower limb complications in diabetic patients. *Journal of Diabetes and its Complications*, 12(1), 10 - 17.

Embil, J.M. 2000. The management of diabetic foot osteomyelitis. *The Diabetic Foot*, 3(3), 76 - 84.

Enderle, M.D., Coerper, S, Schweitzer, H.P., Kopp, A., Thelen., M., Meisner, C., Pressler, H., Becker, H., Cluassen, C., Häring, H. and Luft, D., 1999. Correlation of imaging techniques to histopathology in patients with diabetic foot syndrome and clinical suspicion of osteomyelitis. The role of high resolution ultrasound. *Diabetes Care*, 22(3), 294 - 299.

Eneroth, M., Larrson,J. and Apelqvist, J., 1999. Deep Foot Infections in Patients with Diabetes and Foot Ulcer: An Entity with Different Characteristics, Treatments, and Prognosis. *Journal of Diabetes and Its Complications*, 13 254 - 263.

Ertugrul, M.B., Baktiroglu, S., Salman, S., Unal, S., Askoy, M., Berberoglu, K. and Calangu, S. 2006. The diagnosis of Osteomyelitis of the foot in diabetes: microbiological; examination vs. magnetic resonance imaging and labelled leucocyte scanning. *Diabetic Medicine*. 23, 649-653.

Expert panel, 1994. The problem of sepsis. *Intensive Care Medicine*. 20, 300 - 304.

Faglia, E., Favales, F., Quarantiello, A., Calia, P., Clelia, P., Brambilla, G., Ramplodi, A and Morabito, A., 1998. Angiographic Evaluation of Peripheral Arterial Occlusive Disease and Its Role as a Prognostic Determinant for Major Amputation in Diabetic Subjects With Foot Ulcers. *Diabetes Care*, 21(4), 625 - 630.

Fielding, P.A., Rees, J.I., Bartley, L.E., Facey, P.E. and Jones, J., 2006. The role of SPECT/CT in the evaluation of the painful ankle and foot. *Nuclear Medicine Communications*, 27(3), 299.

Fleck, A., and Meyers M. 1995. Cellular aspects of clinical biochemistry. In: Marshall, W.J. and Bangert, S.K., eds. *Clinical Biochemistry Metabolic and Clinical Aspects*. London Churchill Livingstone, 1995.

Fleischer, A., Didyk, A., Woods, J., Burns, S., Wrobel, J. and Armstrong, D., 2009. Combined Clinical and Laboratory Testing Improves Diagnostic accuracy for Osteomyelitis in the Diabetic Foot. *The Journal of Foot and Ankle Surgery*. 48(1), 39 – 46.

Flemming, D.J., Murphey, M.D and McCarthy, K., 2005. Imaging the foot and ankle: summary and update. *Current Opinions in Orthopaedics*, 16, 54 - 59.

Forouzandeh, F., Aziz Ahari, A., Abolhasani, F. and Larijani, A., 2005. Comparison of different screening tests for detecting diabetic foot neuropathy. *Acta Neurologica Scandinavica*, 112, 409 - 413.

Fosse, S., Hartemann-Heurtier, A., Jacqueminet, S., Ha Van, G., Grimaldi, A. and Fagot-Campagna, A., 2009. Incidence and characteristics of lower limb amputations in people with diabetes. *Diabetic Medicine*. 26, 391 - 396.

Fox, C. and MacKinnon, M., 2002. *Vital Diabetes*. 2<sup>nd</sup> edition London: Class Health.

Frederickson, B., Yuan, H. and Olans R., 1978. Manangement and outcome of pyogenic vertebral osteomyelitis. *Clinical Orthopaedics*, 131, 160 – 167.

Gale, E., 2002. The rise childhood diabetes in the 20<sup>th</sup> century. *Diabetes*, 293, 217 - 228.

Gemmell, H. and Staff, R. Single-photon emission-computed tomography (SPECT). *In: P. Sharp, H. Gemmell and F. Smith, eds. Practical Nuclear Medicine*. Oxford: Oxford University Press, 1998, p13.

Gendrel, D., Assicot, M. and Raymond, J., 1996. Procalcitonin as a marker for the early diagnosis of neonatal bacterial infection. *Journal of Paediatrics*, 128, 570 - 573.

Gendrel, D., Raymond, J. and Assicot, M., 1997. Procalcitonin in bacterial and viral meningitis in children. *Clinical Infectious Diseases*, 24, 1240 - 1242.

Gendrel, D., Raymond, J. and Coste, J., 1999. Comparison of Procalcitonin with C-reactive protein, interleukin 6 and interferon-alpha for differentiation of bacterial vs. viral infections. *Pediatric Infectious Diseases*, 18(10), 875 - 881.

Giurato, L and Uccioli, L., 2006. The diabetic foot: Charcot joint and osteomyelitis. *Nuclear Medicine Communications*, 27(9), 745 - 749.

Grayson, M.L., Gibbons, G.W., Balogh, K., Levin, E and Karchmer, A., 1995. Probing to bone in infected pedal ulcers: a clinical sign of underlying osteomyelitis. *Journal of the American Medical Association*, 273, 721 - 723.

Gürlek, A., Bayraktar, M., Savas, C. and Gedik, O. 1998. Amputation rate in 147 Turkish patients with diabetic foot The Hacettepe University Hospital Experience. *Experimental and Clinical Endocrinology and Diabetes*. 106, 404 – 409.

Haas, D.W. and McAndrew, M.P., 1996. Bacterial Osteomyelitis in Adults: Evolving Considerations in Diagnosis and Treatment. *The American Journal of Medicine*, 101, 550 - 561.

Hartemann-Huertier, A. and Senneville, E., 2008. Diabetic foot osteomyelitis. *Diabetes and Metabolism*, 34, 87 - 95.

Henke, P., Blackburn, S., Wainess, R., Cowan, J., Terando, A., Proctor, M., Wakefield, T., Upchurch, G., Jones, Stanley, J. and Greenfield, L. 2005. Osteomyelitis of the Foot and Toe in Adults Is a Surgical Disease Conservative management worsens lower extremity salvage. *Annals of Surgery*. 241(6), 885 – 894.

Hiew, T.M., Tan, A.M. and Cheng, H.K., 1992. Clinical features and haematological indices of bacterial infections in young infants. *Singapore Medical Journal*, 33, 125 - 130.

Hill, S.L., Holtzman, G.I., and Buse, R., 1999. The Effects of Peripheral Vascular Disease with Osteomyelitis in the Diabetic Foot. *American Journal of Surgery*, 177, 282 - 286.

Hill-Briggs, F., 2003. Problem Solving in Diabetes Self-Management: A Model of Chronic Illness Self-Management Behavior. *Annals of Behavioral Medicine*, 25(3), 182 - 193.



Hladik, M., Olosová, A., Neiser, J. and Zaoral, T. 2005. Procalcitonin –a marker and mediator of inflammation. *Acta chirurgiae plasticae*, 47(2), 51 – 54.

Ho, D., ([davidson.ho@bristol.ac.uk](mailto:davidson.ho@bristol.ac.uk)) 2006. *Lower limb complications for diabetic patients – South-West*. 11 October 2006. Email to: Kim Harman ([kim.harman@ruh-bath.swest.nhs.uk](mailto:kim.harman@ruh-bath.swest.nhs.uk))

Holewski, J., Stess, R., Graf, P. and Grunfeld, C., 1988. Aesthesiometry: Quantification of cutaneous pressure sensation in diabetic peripheral neuropathy. *Journal of Rehabilitation Research and Development*, 25(2), 1 -10.

Jackson, M.A. and Nelson, J.D., 1982. Etiology and medical management of acute suppurative bone and joint infections in paediatric patients. *Journal of Peadiatric Orthopaedics*, 2, 313 - 323.

Jeandrot, A., Richard, J-L., Combescure, C., Jourdan, N., Finge, S., Rodier, M., Corbeau, P., Sotto, A., and Lavinge, J-L., 2008. Serum Procalcitonin and C-reactive protein concentrations to distinguish mildly infected from non-infected diabetic foot ulcers; a pilot study. *Diabetologia*, 51, 347 - 352.

Jeffcoate, W. and Game, F., 2006. The Description and Classification of Diabetic Foot Lesions: Systems for Clinical Care, for Research and for Audit. In: A. Boulton, P. Cavanagh, G Rayman Eds. *The Foot in Diabetes*. John Wiley and Sons Chichester 4<sup>th</sup> ed. 2006 pp92 - 107.

Jeffcoate, W. and Harding, K., 2003. Diabetic foot ulcers. *The Lancet*, 361, 1545 - 1551.

Jeffcoate, W. and Lipsky, B., 2004. Controversies in Imaging and Managing Osteomyelitis of the Foot in Diabetes. *Clinical Infectious Diseases*, Suppl 239 S115 - 122.

Jeffcoate, W., Lipsky, B., Berendt, A., Cavanagh, P., Bus, S., Peters, E., van Houtum, W., Valk, G., and Bakker, K., 2008. Unresolved issues in the management of ulcers of the foot in diabetes. *Diabetic Medicine*. 25, 1380 – 1389.

Jeffcoate, W. and van Houtum, W.H., 2004. Amputation as a marker of the quality of foot care in diabetes. *Diabetologia*. 47, 2051 – 2058.

Jones, L., Chalmers, A., and Dunlop, D., 2001. A Fab' way to resolve diagnostic dilemmas. *Nuclear Medicine Communications*, 22, 346.

Jones, N., Anderson, D. and Stiles, P., 1987. Osteomyelitis in a General Hospital A five year study showing an increase in subacute osteomyelitis. *The Journal of Bone and Joint Surgery (Br)*, 69B(5), 779 - 783.

Jude, E.B., Selby, P.L. and Mawer, E.B., 2002. Inflammatory and bone turnover markers in Charcot arthropathy and osteomyelitis of the feet in diabetic patients [abstract]. *Diabetologia*, (suppl 2). A341 - 342.

Kald, A., Carlsson, R. and Nilsson, E., 1989. Major amputation in a defined population: incidence, mortality and results of treatment. *The British Journal of Surgery*. 76, 308 – 310.

Kaleta J.L., Fleiscli, J.W and Reilly C.H., 2001. The Diagnosis of Osteomyelitis in diabetes Using Erythrocyte Sedimentation Rate A Pilot study. *Journal of the American Podiatric Medical Association*, 91(9), 445 - 450.

Kallio, M.J., Unlika-Kallio, L., Aalto, K., and Petola, H., 1997. Serum C\_Reactive protein, Erythrocyte Sedimentation Rate and White Blood Cell Count in Septic Arthritis of Children. *The Pediatric Infectious Disease Journal*, 16(4), 411 - 413.

Kapoor, A., Page, S., La Valley, M., Gale, D., and Felson, D., 2007. Magnetic Resonance Imaging for Diagnosing Foot Osteomyelitis A meta-analysis. *Archives of Internal Medicine*, 167, 125 - 132.

Katsaros, T., Makras, P., Koutmos, S., Nikolakopoulou, Z., Lymperopoulos, K., Panagiotou, P., Angellou, A., Papadogias, D., Kounadi, T., Bougiouklis, D. and Piadtis, G., 2008. Simple laboratory and clinical tests may be use as indicators of increased suspicion of underlying osteomyelitis (OM) of metatarsals and proximally in the infected diabetic foot (DF). Abstract poster 1053 Diabetes UK Annual professional conference.

Keidar, Z., Militianu, D., Melamed, E., Bar-Shalom, R. and Israel, O., 2005. The Diabetic Foot: Initial Experience with  $^{18}\text{F}$ -FDG PET/CT. *The Journal of Nuclear Medicine*, 46(3), 444 - 449.

Kemp, H., Jackson, J., and Jeremiah, J., 1973. Pyogenic infections occurring primarily in intervertebral discs. *Journal of Bone and Joint Surgery*, 55-B, 698 - 714.

Khachatourians, A.G., Patzakis, M.J., Roidis, N. and Holtom, P.D., 2003. Laboratory Monitoring in Pediatric Acute Osteomyelitis and Septic Arthritis *Clinical Orthopaedics and Related Research*, April (49), 86 - 194.

Kofoed, K., Andersen, O., Kronborg, G., Tvede, M., Petersen, J., Eugen-Olsen, J. and Larsen, K. 2007. Use of plasma C-reactive protein, Procalcitonin, neutrophils, macrophage inhibitory factor, soluble urokinase-type plasminogen activator receptor, and soluble triggering receptor expressed on myeloid cells-1 in combination to diagnose infections: a prospective study. *Critical care*. 11(2), R38 – R48.

Kraus, O., Neufang, A., Eckardt, A., Kustner, E., Beyer, J. and Kann, P., 2002. Interdisciplinary diagnostic and therapeutic approach in diabetic foot syndrome complicated by ischaemia and osteomyelitis. *Medizinische Klinik*, 97(4), 244 - 255.

Krentz, A.J. and Bailey, C.J., 2001. *Type 2 Diabetes in Practice*. London: Royal Society of Medicine Press Ltd.

Larkin, J.G., Frier, B.M. and Ireland, J.T., 1985. Diabetes mellitus and infection. *Postgraduate Medical Journal*, 61 233 - 237.

Lavery, L. A., Armstrong, D.G and Harkless, L.A., 1996. Classification of diabetic foot wounds. *The Journal of Foot and Ankle Surgery*, 5(6), 528 - 531.

Lavery, L.A. Armstrong, D.G. and Murdoch, D.P., 2007. Validation of the Infectious Diseases Society of America's Diabetic Foot Infection Classification System. *Clinical Infectious Diseases*, 44, 562 - 565.

Lavery, L.A. Armstrong, D.G., Peters, E. and Lipsky, B., 2007. Probe-to-Bone Test for Diagnosing Diabetic Foot Osteomyelitis: reliable or relic. *Diabetes Care*, 30, 270 - 274.

Lavery, L.A., Armstrong, D.G., Wunderlich, R.P., Mohler, J., Wendel, C. and Lipsky, B., 2006. Risk Factors for Foot Infections in Individuals With Diabetes. *Diabetes Care*, 29(6), 1288 - 1293.

Lavery, L.A., Armstrong, D.G., Quebedeaux, L. and Walker, S., 1996. Puncture Wounds: Normal Laboratory Values in the Face of Severe Infection in Diabetics and Non-diabetics. *The American Journal of Medicine*, 101, 521 -525.

Lavery, L.A., Peters, E., Armstrong, D., Wendel, C., Murdoch, D and Lipsky, B. Risk factors for developing Osteomyelitis in patients with diabetic foot wounds. *Diabetes Research and Clinical Practice*. (2008) doi:10.1016/j.diabres.2008.11.030

Lawlor, D., Davey-Smith, G., Rumley, A., Lowe, G. and Shah, E., 2005. Associations of fibrinogen and C-reactive protein with prevalent and incident

coronary heart disease are attenuated by adjustment for confounding factors. *Thrombosis and Haemostasis*, 93, 955 - 963.

Leese, G., Reid, F., Green, V., MacAlpine, R., Cunningham, S., Emslie-Smith, A., Morris, A., McMurray, B. and Connacher, A.. 2006. Stratification of foot ulcer risk in patients with diabetes: a population based study. *International Journal of Clinical Practice*, 60, (5), 541 - 545.

Leese, G. 2007. The value of evidence-based foot screening in diabetes in the primary care setting. *Diabetes and Primary Care*. 9(6) 318 - 322.

Leese, G., Schofield, C., McMurray, B., Libby, G., Golden, J., MacAlpine, R., Cunningham, S., Morris, A., Flett, M. and Griffiths, G. 2007. Scottish Foot Ulcer Risk Score Predicts Foot Ulcer Healing in a Regional Specialist Foot Clinic. *Diabetes Care*, 30, (8), 2064 - 2069.

LeFrock, J. and Joseph, W., 1995. Bone and soft-tissue infections of the lower extremity in diabetes. *Clinical Podiatric Medicine and Surgery*, 12, 87 -103.

Leichter, S.B., Allweiss, P. Harley, J., Clay, J., Kuperstein-Chase, J., Sweeney, G. and Kolkin, J., 1998. Clinical characteristics of diabetic patients with serious pedal infections. *Metabolism*, 37 (Suppl 1) (22), 22 - 24.

Lew, D.P. and Waldvogel, F.A., 1999. Use of Quinolones in Osteomyelitis and Infected Orthopaedic Prostheses. *Drugs*, 58 (suppl.2), 85 - 91.

Lewis, V.L.Jr., Bailey, M.H., Pulawski, G., Kind, G., Bashioum, R. and Hendrix, R., 1998. The diagnosis of osteomyelitis in patients with pressure sores. *Plastic and Reconstructive Surgery*, 81(229), 229 - 232.

Li, S.F., Henderson, J., Dickman, E. and Darzynkiewicz, R., 2004. Laboratory Tests in Adults with Monoarticular Arthritis: Can They Rule Out a Septic Joint? *Academic Emergency Medicine*, March 11(3), 276 - 280.

Lipsky, B. 1997. Osteomyelitis of the foot in diabetic patients. *Clinical Infectious Diseases*, 25, 1318 - 1326.

Lipsky, B., Berendt, A.R., Gunner Deery, H., Embil, J., Warren, J., Karrchmer, A., LeFrock, J., Lew, D., Mader, J., Norden, C. and Tan, J., 2004. IDSA Guidelines Diagnosis and Treatment of Diabetic Foot Infections. *Clinical Infectious Diseases*, 39, 885 - 910.

Lipsky, B., Berendt, A., Embil, J., and de Lalla, F., 2004a. Diagnosing and treating diabetic foot infections. *Diabetes Metabolism Research and Reviews*, 20 (Suppl 1), S56 - S64.

Lipsky, B., Pecoraro, R. and Wheat, L., 1990. The Diabetic Foot Soft Tissue and Bone Infection. *Infectious Disease Clinics of North America*, 4(3), 409 -432.

Lipsky, B., Sheehan, P., Armstrong, D., Tice, A., Polis, A. and Abramsom, M., 2007. Clinical predictors of treatment failure for diabetic foot infections: data from a prospective trial. *International Wound Journal*, 4(1), 30 - 38.

Lipsy, B. 2008. Bone of Contention: Diagnosing Diabetic Foot Osteomyelitis. *Clinical Infectious Diseases*. 47, 528 - 530.

Longjohn, D.B., Zions, L.E. and Stott, N., 1995. Acute Haemtogenous Osteomyelitis of the Epiphysis. *Clinical orthopaedics and related research*, 316, 227 - 234.

Macfarlane, R.M. and Jeffcoate, W., 1997. Factors contributing to the presentation of diabetic foot ulcers. *Diabetic Medicine*, 14. 867 - 870.

Macfarlane, R.M. and Jeffcoate, W., 1999. Classification of diabetic foot ulcers: the S(AD) SAD system. *Diabetic Foot*, 2(4), 123 - 131.

MacRury, S., Lockhart, J., Small, M., Weir, A., MacCuish, A. and Lowe, G., 1990. Do Rheological Variables Play a Role in Diabetic Peripheral Neuropathy? *Diabetic Medicine*, 8, 232 - 236.

Mader, J.T., Shirtliff, M. and Calhoun, J.H., 1997. Staging and Staging Application in Osteomyelitis. *Clinical Infectious Diseases*, 25, 1303 - 1309.

Mader, J.T., Cripps, M.W. and Calhoun, J.H., 1999. Adult Posttraumatic Osteomyelitis of the Tibia. *Clinical orthopaedics and related research*, 360, 14 - 21.

Mandell, G.A., 1996. Imaging in the Diagnosis of Musculoskeletal Infections in Children. *Current Problems in Pediatrics*. Aug 26, 218 - 237.

Mason, J., O'Keffe, C., Hutchinson, A., McIntosh, A., Young, R., and Booth, A., 1999. A systematic review of foot ulcer patients with Type 2 diabetes mellitus. I: prevention. *Diabetic Medicine*, 16, 801 - 812.

Mason, J., O'Keffe, C., Hutchinson, A., McIntosh, A., Young, R., and Booth, A., 1999a. A systematic review of foot ulcer patients with Type 2 diabetes mellitus. II: Treatment. *Diabetic Medicine*, 16, 889 - 909.

Mawdsley, R., Behm-Pugh, A., Campbell, J., Carroll, C., Chernikovich, K., Mowbray, M. and Spagnuolo, T., 2004. Reliability of Measurements with Semmes-Weinstein Monofilaments in Individuals with Diabetes. *Physical and Occupational Therapy in Geriatrics*, 22(3), 19 - 36.

Meisner, M. 2000. *Procalcitonin (PCT) A new, innovative infection parameter Biochemical and clinical aspects*. 3<sup>rd</sup> edition., Stuttgart, Thieme.

Meisner, M. 2005. Biomarkers of sepsis: clinically useful? *Current Opinions in Critical Care*. 11, 473 - 480.

McGill, M., Molyneaux, L., Spencer, R., Heng, L. and Yue, D., 1999. Possible Sources of Discrepancies in the Use of the Semmes-Weinstein Monofilament. *Diabetes Care*, 22(4), 598 - 602.

McNeely, M., Boyko, E., Ahroni, J., Stensel, V., Reiber, G., Smith, D. and Pecoraro, R., 1995. The Independent Contributions of Diabetic Neuropathy and Vasculopathy in Foot Ulceration How great are the risks? *Diabetes Care*, 18(2), 216 - 219.

Meijer, J., van Sonderen, E., Blaauwwekel, E., Smit, A., Groothoff, J., Eisma, W. and Links, T., 2000. Diabetic Neuropathy Examination A hierarchical scoring system to diagnose distal polyneuropathy in diabetes. *Diabetes Care*, 23(6), 750 - 753.

Miranda-Palma, B., Sosensko, J., Bowker, J., Mizel, M. and Boulton, A.J.M., 2005. A comparison of the monofilament with other testing modalities for foot ulcer susceptibility. *Diabetes Research and Clinical Practice*, 70, 8 - 12.

Molenar, D, Palumbo, P. Wilson, W. and Ritts, R., 1976. Leukocyte chemotaxis in diabetic patients and their first degree relatives. *Diabetes, suppl. 2*, 25, 880.

Morrison, W.B., Schweitzer, M.E., Wapner, K.L., Hecht, P.J., Gannon, F.H., and Behm, W.R., 1995. Osteomyelitis in Feet of Diabetics: Clinical Accuracy, Surgical Utility, and Cost-Effectiveness of MR Imaging. *Radiology*. 196(2), 557 – 564.

Morrison, W.B., Schweitzer, M.E., Batte, W., G., Radack, D.P., and Russel, K.M. Osteomyelitis of the Foot: Relative Importance of Primary and Secondary MR Imaging Signs. *Radiology*. 207(3), 625 – 632.



Müller, B., White, J., Nylen, E., Snider, R., Becker, K. and Habener, J.. 2008. Ubiquitous Expression of the Calcitonin-I Gene in Multiple Tissues in Response to Sepsis. *The Journal of Clinical Endocrinology and Metabolism*. 86(1), 396 - 404.

National Health Service National Diabetes Support Team, 2008. *Improving Emergency and Inpatient Care for People with Diabetes*. London: NHS Diabetes.

National Kidney Federation, 2007. *UK NKF news* [on line]. Available at: <<http://www.kidney.org.uk/Medical-Info/ckd-info/calc-gfr.html>> [Accessed 17March 2007].

Nesto, R., 2004. C-reactive protein, its role in inflammation, Type 2 diabetes and cardiovascular disease, and the effects of Insulin-sensitizing treatment with thiazolidinediones. *Diabetic Medicine*, 21(8), 810 - 817.

New, J., McDowell, D., Burns, E. and Young, R. 1998. Problem of Amputations in Patients with Newly Diagnosed Diabetes Mellitus. *Diabetic Medicine*. 15, 760 -764.

Newman, L.G., Waller, J. Palestro, C.J., Schwartz, M., Klein, M., Hermann, G., Harrington, E., Harrington, M., Roman, S. and Stagnaro-Green A.,1991. Unsuspected osteomyelitis in diabetic foot ulcers: diagnosis and monitoring by leukocyte scanning with indium 111 oxyquinolone. *Journal of the American Podiatric Association*, 266, 1246 - 1251.

Newman, L.G., Waller, J. Palestro, C.J., Schwartz, M., Klein, M., Hermann, G., Harrington, E., Harrington, M., Roman, S. and Stagnaro-Green A. 1992. Leucocyte scanning with 111n is superior to magnetic resonance imaging in diagnosis of clinically unsuspected osteomyelitis in diabetic foot ulcers. *Diabetes Care*. 15, 1527-1530.

NHS Centre for reviews and dissemination, 1999. Effective healthcare; complications of diabetes: screening for retinopathy and management of foot ulcers. 5(4).

Nather, A., Bee, c., Huak, C., Chew, J., Lin, C., Neo, S. and Sim, E.. 2008. Epidemiology of diabetic foot problems and predictive factors for limb loss. *Journal of Diabetes and Its Complications*. 22 77 – 82.

Olmus, P., Cataland, S., O'Dorisio, T., Casey, C., Smead, W. and Sheldon, S., 1995. The Semmes-Weinstein Monofilament as a Potential Predictor of Foot Ulceration in Patients With Non Insulin-Dependent Diabetes. *American Journal of the Medical Sciences*, 309(2), 76 - 82.

O'Mara, N. 2008. Anemia in Patients With Chronic Kidney Disease. *Diabetes Spectrum*. 21(1), 12 - 19.

O'Neill, J., Mccann, S. and Lagan, K., 2006. Tuning fork (128Hz) versus Neurothesiometer: a comparison of methods of assessing vibration sensation in patients with diabetes mellitus. *International Journal of Clinical Practice*, 60(2), 174 - 178.

Oncul, O., Yildiz, S. Gurer, U., Yeniiz, E., Qyrdedi, T., Top, T., Gocer, P., Akarsu, B., Cevikbas, A. and Cavuslu, S., 2006. Effect of the function of polymorphonuclear leukocytes and interleukin-1 beta on wound healing in patients with diabetic foot infections. [on line] *Journal of Infection*. Available at <<http://www.elsevier.health.com/journals/jinf>> [accessed 28 July 2006].

Otremeski, I., Newman, R., Kahn, P., Stadler, J., Kariv, N., Skornik, Y. and Goldman, G., 1993. Leukergy – a new diagnostic test for bone infection. *The Journal of Bone and Joint Surgery (Br)*, 75B(5), 734 - 736.

Paluska, S., 2004. Osteomyelitis. *Clinics in Family Practice*. 6(1), 127- 156.

Pepys, M., 1981. C Reactive Protein fifty years on. *The Lancet*, March 21. 653 - 657.

Perry, M., 1996. Erythrocyte sedimentation rate and C-reactive protein in the assessment of suspected bone infection – are they reliable indicators? *The Royal College of Surgeons of Edinburgh*, 41, April, 116 - 119.

Pittet, D., Wyassa, B., Heter-Clavel, C., Kursteiner, K., Vaucher, J. and Lew, D., 1999. Outcome of diabetic foot infections treated conservatively: a retrospective cohort study with long term follow up. *Archives of Internal Medicine*, 159, 851 - 856.

Petola, H., 1982. C Reactive Protein for rapid monitoring of infections of the Central Nervous System. *The Lancet*, May 1. 980 - 983.

Petola, H. and Räsänen, J.A., 1982. Quantitative C-reactive protein in relation to erythrocyte sedimentation rate, fever, and duration of antimicrobial therapy in bacteremic diseases of childhood. *Journal of Infection*, 5, 257 - 267.

Plank, M., Graham, P. and Hyer, S., 2000. Clinical implications of foot pressures during normal gait. *The Diabetic Foot*, 3(2) 100 -104.

Prompers, L., Huijberts, M., Schaper, N., Apelqvist, J., Bakker, K., Edmonds, M., Holstein, P., Jude, E., Jirkovska, A., Mauricio, D., Piaggese, A., Reike, H., Spraul, M., Van Acker, K., Van Baal, S., Van Merode, F., Uccioli, L., Urbancic, V. and

Ragnarson-Tennvall, G. 2008. Resource utilisation and costs associated with the treatment of diabetic foot ulcers. Prospective data from the Eurodiale study. *Diabetologia*. 51, 1826 -1834.

Ragnarson, T.G. and Apelqvist, J. 2004. Health-economic consequences of diabetic foot lesions. *Clinical Infectious Diseases*. 39 Suppl2 S132 - S139.

Rayman, G., Krishnan, S., Baker, N., Wareham, A. and Rayman, A., 2004. Are We Underestimating Diabetes-Related Lower-Extremity Amputation Rates? *Diabetes Care*, 27(8), 1882 -1896.

Reiber, G., Boyko, E.J. and Smith, D.G., 1995. Lower extremity foot ulcers and amputations in individuals with diabetes. In: Harris M., ed. *Diabetes in America*. Bethesda: National Institutes of Health publication No 95-1468, 1995, pp409 - 428.

Reiber, G., 1996. The Epidemiology of Diabetic Foot Problems. *Diabetic Medicine*, 13, S6 - S11.

Reiber G., Lipsky B.A., and Gibbons G.W., 1998. The Burden of Diabetic Foot Ulcers. *American Journal of Surgery*, 176 (Suppl 2A), 5S - 10S.

Reinhart,K., Karzai, W. and Meisner, M., 2000. Procalcitonin as a marker of the systemic inflammatory response to infection. *Intensive Care Medicine*, 26, 1193 - 1200.

Roglic, G. and Unwin., N., 2010. Mortality attributable to diabetes: Estimates for the year 2010. *Diabetes Research and Clinical Practice*. 87, 15-19.

Roine, I., Arguedas, A., and Faingezicht, I.,1997. Early detection of sequele-prone osteomyelitis in children with use of simple clinical and laboratory criteria. *Clinical Infectious Diseases*, 24, 849 - 853.

Rozzanigo, U., Tagliani, A., Vittorini, E., Pacchioni, R., Renzi Brivio, L. and Caudana, R.. Role of magnetic resonance imaging in the evaluation of diabetic foot with suspected osteomyelitis. *Musculoskeletal Radiology*. (2008). doi 10.1007/s11547-008-0337-7.

Rubello, D., Casara, D., Maran, A., Avogaro, A., Tiengo, A. and Muzzio, P., 2004. Role of anti-granulocyte Fab' fragment antibody scintigraphy (LeukoScan) in evaluating bone infection: acquisition protocol, interpretation criteria and clinical results. *Nuclear Medicine Communications*, 25, 39 - 47.

Sannomiya, P., Pereira, M. and Garcia-Leme, J., 1990. Inhibition of leukocyte chemotaxis by serum factor in diabetes mellitus: Selective depression of cell responses mediated by complement-derived chemoattractants. *Agents and Actions*, 30, 369 - 376.

Sartoris, D., 1994. Cross-Sectional Imaging of the Diabetic Foot. *The Journal of Foot and Ankle*. 33(6), 531 - 545.

Sattar, N., 2006. High sensitivity C-reactive protein in cardiovascular disease and type 2 diabetes: evidence for a clinical role? *The British Journal of Diabetes and Vascular Disease*, 6(1), 5 - 8.

Sattar, N., 2006a ([nsattar@clinmed.gla.ac.uk](mailto:nsattar@clinmed.gla.ac.uk)) CRP or hsCRP IN DIABETES 08 March 2006 Email to: Kim Harman ([kim.harman@ruh-bath.swest.nhs.uk](mailto:kim.harman@ruh-bath.swest.nhs.uk))

Schaper, N. 2004. Diabetic foot ulcer classification system for research purposes: a progress report on criteria for including patients in studies. *Diabetes Metabolism Research and Reviews*. 20(Suppl 1) S90-S95.

Schofield, C.J., Libby, G., Brennan, G.M., MacAlpine, R.R., Morris, A.D. and Leese, G. 2006. Mortality and Hospitalization in Patients After Amputation. *Diabetes Care*. 29(10) 2252 - 2256.

Senneville, E., Melliez, H., Beltrand, E., Legout, L., Valette, M., Cazaubiel, M., Cordonnier, M., Cailaux, M., Yazdanpanah, Y. and Mouton, Y. 2006. Culture of percutaneous bone biopsy specimens for diabetic foot osteomyelitis: concordance with ulcer swab cultures. *Clinical Infectious Diseases*. 45, 57 - 62.

Sharp, P., 1998. Nuclear Medicine Imaging. *In: P. Sharp, H. Gemmell, and F. Smith, eds. Practical Nuclear Medicine.* Oxford: Oxford University Press, 1998, p1.

Shaw, J., Sicree, R. and Zimmet, P. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice.* 87, 4-14.

Shone, A., Burnside, J., Chipchase, S., Game, F. and Jeffcoate, W., 2006. *Probing the Validity of the Probe-to-Bone Test in the Diagnosis of Osteomyelitis of the Foot in Diabetes.* *Diabetes Care,* 29(4), 945.

Simon, L., Gauvin, F., Amre, D., Saint\_louis, P and Lacroix J.. 2004. Serum Procalcitonin and C-Reactive Protein Levels as Markers of Bacterial Infection: A Systematic Review and Meta-Analysis. *Clinical Infectious Diseases.* 39, 206-217. (Also see erratum *Clinical Infectious Diseases.* 40, 1386 -1388.

Singh, N., Armstrong, D. and Lipsky, B., 2005. Preventing foot ulcers in people with diabetes. *Journal of the American Medical Association,* 293, 217 -228.

Sitter, T., Schmidt, M., Schneider, S. and Schiffel, H. 2002. Differential diagnosis of bacterial infection and inflammatory response in kidney disease using Procalcitonin. *Journal of Nephrology.* 15(3), 297-301.

Slovenkai, M.P., 1998. Foot problems in diabetes. *Medical Clinics of North America,* 82 (4), 949 - 970.

Smith, F. 1998. The skeletal system. *In: P. Sharp, H. Gemmell and F. Smith, eds. Practical Nuclear Medicine.* Oxford: Oxford University Press, 1998, p235.

Snyder, R., Cohen, M. and Sun, C., 2001. Osteomyelitis in the Diabetic Patient: Diagnosis and Treatment Part 1: Overview, Diagnosis, and microbiology. *Ostomy Wound Management,* 47, 18 -30.

Springett, K. and White, R., 2002. Skin changes in the 'at risk' foot and their treatment. *British Journal of Community Nursing*, Dec. 25-32.

Stevens, P.E., O'Donoghue, D.J. and Lameire, N.R., (2003). Anaemia in patients with diabetes: Unrecognised, undetected and untreated? *Current Medical Research and Opinion*, 19(5), 395-401.

Stuart, L., Wiles, P., and Chadwick, P., 2004. Improving peripheral arterial assessment of people with diabetes. *The Diabetic Foot*, 7(4), 183 -185.

Stucker, F., Herrmann, F., Graf, J-D., Michel, J-P., Krause, K. and Gavazzi, G. 2005. Procalcitonin and Infection in Elderly Patients. *Journal of the American Geriatrics Society*. 53(8), 1392 -1395.

Sutton, P., Harley, J., Jacobson, A., Lipsky, B. 2000. Diagnosing osteomyelitis with percutaneous bone biopsy in patients with diabetes and foot infection. In: Alexandria V. Ed. *Infectious Diseases Society of America*.

Tan, J., Anderson, J, Watanakunakorn, C., and Phair, J., 1975. Neutrophil dysfunction in diabetes mellitus. *Journal of Laboratory Clinical Medicine*, 85, 26 - 30.

Tan, J. and File, T., 1999. Diagnosis and treatment of diabetic foot infections. *Ballière's Clinical Rheumatology*, 13(1), 149 - 161.

Tan, P. and Teh, J. 2007. MRI of the diabetic foot: differentiation of infection from neuropathic change. *British Journal of Radiology*. 80(959) 939 - 948.

Teh, J., Berendt, A., and Lipsky, B.A. 2010. Investigating suspected bone infection in the diabetic foot. *British Medical Journal*. February, Vol. 340 415-417.

- Tentolouris, N., Al-Sabbagh, S., Walker, M.G., Boulton, A.J.M., Jude, E. 2004. Mortality in Diabetic and Nondiabetic Patients After Amputations Performed From 1990 to 1995. *Diabetes Care*. 27(7) 1598 - 1604.
- Thayyil, S., Shenoy, M., Hamaluba, M., Gupta, A., Frater, J and Verber, I. 2005. Is Procalcitonin useful in early diagnosis of serious bacterial infections in children? *Acta Paediatrica*. 94, 155 -158.
- Thomas, M., MacIssac, R., Tsalamandris, C., Power, D and Jerums, G., 2003. Unrecognized Anemia in Patients With Diabetes. *Diabetes Care*, 26 (4), 1164 - 1169.
- Thomas, S. and Rampersad, M., 2004. Anaemia in diabetes. *Acta Diabetologia*, 41, S13 - S17.
- Thomson, M., Paisey, R., Frost, J., Potter, J., Taylor, P. and Bower, L., 2001. Sensory testing of feet in subjects without diabetes as a baseline for neuropathy studies. *British Journal of Podiatry*, 4(2), 49 - 52.
- Toursarkissian, B., Shireman, P.K., Harrison, A., D'Ayala, M., Schoolfield, J. and Sykes, M.T., 2002. Major lower-extremity amputation: contemporary experience in a single Veterans Affairs institution. *The American Journal of Surgery*. 68, 606 – 610.
- Treece, K.A., Macfarlane, R.M., Pound, N., Game, F. and Jeffcoate, W., 2004. Validation of a system of foot ulcer classification in diabetes mellitus. *Diabetic Medicine*, 21, 987 - 991.
- Tumeh, S.S. and Tohmeh, A.G., 1991. Nuclear Medicine Techniques in Septic Arthritis and Osteomyelitis, *Clinical Orthopaedics*, 17, 559-83 In: Paluska, S., 2004. Osteomyelitis. *Clinics in Family Practice*. 6(1), 127 - 156.



UK Data Archive 2006. *Health Survey of England 2004*. [on line] Available at: <[URL:http://www.uic.nhs.uk/services/qof/documents/QoF0405\\_PCTs-Prevalence.xls/view?searchterm=diabetes%20prevalence](http://www.uic.nhs.uk/services/qof/documents/QoF0405_PCTs-Prevalence.xls/view?searchterm=diabetes%20prevalence)> [Accessed 11 October 2006]

UKPDS 33, 1998. Intensive blood glucose control with sulphonylurea or Insulin compared with conventional treatment and the risk of complications in type 2 diabetes. *The Lancet*. 352, 837 - 853.

Unkila-Kallio, L., Kallio, M.J, Eskola, J. and Petola, H., 1994. Serum C-Reactive Protein, Erythrocyte Sedimentation Rate, and White Blood Cell Count in Acute Haemogenous Osteomyelitis of Children. *Pediatrics*, 93(1), 59 - 62.

Unkila-Kallio, L., Kallio, M.L. and Petola, H., 1994. The Usefulness of C-Reactive protein IN THE Identification of Concurrent Septic Arthritis in Children Who Have Acute Hematogenous Osteomyelitis. *The Journal of Bone and Joint Surgery (Am)*, 76-A(6), 848 - 853.

Upchurch, G.R., Keagy, B.A. and Johnson, G., 1997. An acute phase reaction in diabetic patients with foot ulcers. *Cardiovascular surgery*, 5(1), 32 - 36.

Uzun, G., Solmazgul, E., Curuksulu, H., Turhan, V., Ardic, N., Top, C., Yildiz, S. and Cimsit, M. 2007. *Tohoku Journal of Experimental Medicine*. 213(4), 305 - 312.

Vamos, E.P., Bottle, A., Majeed, A. and Millett, C. 2010. Trends in lower extremity amputations in people with and without diabetes in England, 1996-2005. *Diabetes Research and Clinical Practice*. 87, 275 – 282.

Waldvogel, F.A., Medoff, G. and Swartz, M.N., 1970. Osteomyelitis: a review of clinical features, therapeutic considerations and unusual aspects (first of three parts). *The New England Journal of Medicine*, 282(4), 198 - 206.

Walk, G.D., de Sonnaville, J.J., van Houtum, W.H., Heine, R.J., van Eijk, J.T.M., Bouter, L.M. and Bertelsmann, F.W., 1997. The assessment of diabetic polyneuropathy in daily clinical practice: reproducibility and validity of Semmes Weinstein monofilaments examination and clinical neurological examination. *Muscle and Nerve*, 20, 116 - 118.

Wall, E.J., 1998. Childhood osteomyelitis and septic arthritis. *Current opinions in Pediatrics*, 10, 73 - 76.

Weatherley, B., Chambless, L., Heiss, G., Catellier, D., and Ellison, C., 2006. The reliability of the ankle-brachial index in the Atherosclerosis Risk in communities (ARIC) study and the NHLBI Family Heart Study (FHS). *Cardiovascular Disorders*. 6(7). Available at: <[URL:http://www.biomedcentral.com/1471-2261/6/7](http://www.biomedcentral.com/1471-2261/6/7)> [Accessed 11 October 2006]

Weinstein, D., Wang, A., Chambers, R., Stewart, C. and Motz H. 1993. Evaluation of magnetic resonance imaging in the diagnosis of Osteomyelitis in diabetic foot infections. *Foot and Ankle*. 14, 18-22.

Wheat, L., Allen, S., Henry, M., Kerneck, C., Siders, J., Kuebler, T., Fineberg, N. and Norton, J. 1986. Daibetic foot infections: bacteriologic analysis. *Archives of Physical Medicine and Rehabilitation*, 146, 1935 - 1940.

Wikblad, K.F., Wibell, L.B., and Montin, K.R., 1990. The patient's experience of diabetes and its treatment: construction of an attitude scale by a semantic differential technique. *Journal of Advanced Nursing*, 15, 1983 - 1091.

Wild, S., Roglic, G., Green A., Sicree, R. and King, H., 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27, 1047 - 1053.

Williams, G. and Pickup, J.C., 1999. *Handbook of Diabetes*. Oxford: Blackwell Science Ltd..

Williams, D., Harding, K and Price, P., 2005. An Evaluation of the Efficacy of Methods Used in Screening for Lower-Limb Arterial Disease in Diabetics. *Diabetes Care*, 28(9), 2206 - 2210.

WORLD HEALTH ORGANISATION 1968: *Nutritional Anaemia: Report of a WHO Scientific Group*. Geneva, World Health Organisation.

WORLD HEALTH ORGANISATION Department of Noncommunicable Disease Surveillance General, 1999. *Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications*. (Chairman K.G.M.M. Alberti and P.Z. Zimmet), Geneva.

WORLD HEALTH ORGANISATION 2001. Iron deficiency anaemia assessment, prevention and control A guide for programme managers. Available at: <URL <http://whqlibdoc.who.int/hq/2001/WHO-IUHD-01.3.pdf> > [Accessed 29 October 2009]

Wrobel, J. and Connolly, J., 1998. Making the Diagnosis of Osteomyelitis The Role of Prevalence. *Journal of the American Podiatric Medical Association*, 88(7), 337 - 343.

Wu, J., Gorbachova, T., Morrison, W. and Haims, A. 2007. Imaging\_guided Bone Biopsy for Osteomyelitis: Are There Factors Associated With Positive or negative Cultures? *American Journal of Roentology*. 188, 1529 - 1534.

YHPHO 2009. Diabetes prevalence models available at <URL <http://www.yhpho.org.ukresource/item.aspx?RID=9905> > [accessed 02 December 2009]

Yu, H-I, Sheu, W. and Song, Y-M., 2004. C-reactive protein and risk factors for peripheral vascular disease in subjects with Type 2 diabetes mellitus. *Diabetes Medicine*, 21(4), 336 - 341.

Yuh, W., Corson, J., Baraniewski, H., Rezai, K., Shamma, A., Kathol, M., Sato, Y., El-Khoury, G., Hawes, D., Platz, C., Cooper, R. and Corry, R. 1989. Osteomyelitis of the Foot in Diabetic Patients: Evaluation with Plain Film, <sup>99m</sup>Tc-MDP Bone Scintigraphy, and MR Imaging. *American Journal of Roentgenology*. 152, 795-800.

Zgonis, T. and Roukis, T.S., 2005. A systematic approach to diabetic foot infections. *Advances in Therapy*, 22(3), 244 - 262.

Zlonis, M., 1993. The Mystique of the Erythrocyte Sedimentation Rate a reappraisal of one of the oldest laboratory tests still in use. *Clinics in Laboratory Medicine*. 13(4), 787 - 800.